Factor IX

19. GlycoPEGylation of Factor IX produced in CHO cells

This example sets forth the preparation of asialoFactor IX and its sialylation with CMP-sialic acid-PEG.

Desialylation of rFactor IX. A recombinant form of Coagulation Factor IX (rFactor IX) was made in CHO cells. 6000 IU of rFactor IX were dissolved in a total of 12 mL USP H_2O. This solution was transferred to a Centricon Plus 20, PL-10 centrifugal filter with another 6 mL USP H_2O. The solution was concentrated to 2 mL and then diluted with 15 mL 50 mM Tris-HCl pH 7.4, 0.15 M NaCl, 5 mM $CaCl_2$, 0.05% NaN_3 and then reconcentrated. The dilution/concentration was repeated 4 times to effectively change the buffer to a final volume of 3.0 mL. Of this solution, 2.9 mL (about 29 mg of rFactor IX) was transferred to a small plastic tube and to it was added 530 mU α2-3,6,8-Neuraminidase– agarose conjugate (*Vibrio cholerae*, Calbiochem, 450 µL). The reaction mixture was rotated gently for 26.5 hours at 32 °C. The mixture was centrifuged 2 minutes at 10,000 rpm and the supernatant was collected. The agarose beads (containing neuraminidase) were washed 6 times with 0.5 mL 50 mM Tris-HCl pH 7.12, 1 M NaCl, 0.05% NaN_3. The pooled washings and supernatants were centrifuged again for 2 minutes at 10,000 rpm to remove any residual agarose resin. The pooled, desialylated protein solution was diluted to 19 mL with the same buffer and concentrated down to ~ 2 mL in a Centricon Plus 20 PL-10 centrifugal filter. The solution was twice diluted with 15 mL of 50 mM Tris-HCl pH 7.4, 0.15 M NaCl, 0.05% NaN_3 and reconcentrated to 2 mL. The final desialyated rFactor IX solution was diluted to 3 mL final volume (~10 mg/mL) with the Tris Buffer. Native and desialylated rFactor IX samples were analyzed by IEF-Electrophoresis. Isoelectric Focusing Gels (pH 3-7) were run using 1.5 µL (15 µg) samples first diluted with 10 µL Tris buffer and mixed with 12 µL sample loading buffer. Gels were loaded, run and fixed using standard procedures. Gels were stained with Colloidal Blue Stain (Figure 154), showing a band for desialylated Factor IX.

Preparation of PEG (1 kDa and 10 kDa)-SA-Factor IX. Desialylated rFactor-IX (29 mg, 3 mL) was divided into two 1.5 mL (14.5 mg) samples in two 15 mL centrifuge tubes. Each solution was diluted with 12.67 mL 50 mM Tris-HCl pH 7.4, 0.15 M NaCl, 0.05% NaN_3 and either CMP-SA-PEG-1k or 10k (7.25 µmol) was added. The tubes were

inverted gently to mix and 2.9 U ST3Gal3 (326 µL) was added (total volume 14.5 mL). The tubes were inverted again and rotated gently for 65 hours at 32 °C. The reactions were stopped by freezing at –20 °C. 10 µg samples of the reactions were analyzed by SDS-PAGE. The PEGylated proteins were purified on a Toso Haas Biosep G3000SW (21.5 x 30 cm, 13 um) HPLC column with Dulbecco's Phosphate Buffered Saline, pH 7.1 (Gibco), 6 mL/min. The reaction and purification were monitored using SDS Page and IEF gels. Novex Tris-Glycine 4-20% 1 mm gels were loaded with 10 µL (10 µg) of samples after dilution with 2 µL of 50 mM Tris-HCl, pH 7.4, 150 mM NaCl, 0.05% NaN_3 buffer and mixing with 12 µL sample loading buffer and 1 µL 0.5 M DTT and heated for 6 minutes at 85 °C. Gels were stained with Colloidal Blue Stain (Figure 155) showing a band for PEG (1 kDa and 10 kDa)-SA-Factor IX.

20. Direct Sialyl-GlycoPEGylation of Factor IX

This example sets forth the preparation of sialyl-PEGylation of Factor IX without prior sialidase treatment.

Sialyl-PEGylation of Factor-IX with CMP-SA-PEG-(10 KDa). Factor IX (1100 IU), which was expressed in CHO cells and was fully sialylated, was dissolved in 5 mL of 20 mM histidine, 520 mM glycine, 2% sucrose, 0.05% NaN_3 and 0.01% polysorbate 80, pH 5.0. The CMP-SA-PEG-(10 kDa) (27 mg, 2.5 µmol) was then dissolved in the solution and 1 U of ST3Gal3 was added. The reaction was complete after gently mixing for 28 hours at 32°C. The reaction was analyzed by SDS-PAGE as described by Invitrogen. The product protein was purified on an Amersham Superdex 200 (10 x 300 mm, 13 µm) HPLC column with phosphate buffered saline, pH 7.0 (PBS), 1 mL/min. R_t = 9.5 min.

Sialyl-PEGylation of Factor-IX with CMP-SA-PEG-(20 kDa). Factor IX (1100 IU), which was expressed in CHO cells and was fully sialylated, was dissolved in 5 mL of 20 mM histidine, 520 mM glycine, 2% sucrose, 0.05% NaN_3 and 0.01% polysorbate 80, pH 5.0. The CMP-SA-PEG-(20 kDa) (50 mg, 2.3 µmol) was then dissolved in the solution and CST-II was added. The reaction mixture was complete after gently mixing for 42 hours at 32°C. The reaction was analyzed by SDS-PAGE as described by Invitrogen.

The product protein was purified on an Amersham Superdex 200 (10 x 300 mm, 13 μm) HPLC column with phosphate buffered saline, pH 7.0 (Fisher), 1 mL/min. R_t = 8.6 min.

21. Sialic Acid Capping of GlycoPEGylated Factor IX

This examples sets forth the procedure for sialic acid capping of sialyl-glycoPEGylated peptides. Here, Factor-IX is the exemplary peptide.

Sialic acid capping of N-linked and O-linked Glycans of Factor-IX-SA-PEG (10 kDa). Purified r-Factor-IX-PEG (10 kDa) (2.4 mg) was concentrated in a Centricon® Plus 20 PL-10 (Millipore Corp., Bedford, MA) centrifugal filter and the buffer was changed to 50 mM Tris-HCl pH 7.2, 0.15 M NaCl, 0.05% NaN_3 to a final volume of 1.85 mL. The protein solution was diluted with 372 μL of the same Tris buffer and 7.4 mg CMP-SA (12 μmol) was added as a solid. The solution was inverted gently to mix and 0.1 U ST3Gal1 and 0.1 U ST3Gal3 were added. The reaction mixture was rotated gently for 42 hours at 32 °C.

A 10 μg sample of the reaction was analyzed by SDS-PAGE. Novex Tris-Glycine 4-12% 1 mm gels were performed and stained using Colloidal Blue as described by Invitrogen. Briefly, samples, 10 μL (10 μg), were mixed with 12 μL sample loading buffer and 1 μL 0.5 M DTT and heated for 6 minutes at 85 °C (Figure 156, lane 4).

Factor VIIa

22. GlycoPEGylation of Recombinant Factor VIIa produced in BHK cells

This example sets forth the PEGylation of recombinant Factor VIIa made in BHK cells.

Preparation of Asialo-Factor VIIa. Recombinant Factor VIIa was produced in BHK cells (baby hamster kidney cells). Factor VIIa (14.2 mg) was dissolved at 1 mg/ml in buffer solution (pH 7.4, 0.05 M Tris, 0.15 M NaCl, 0.001 M $CaCl_2$, 0.05% NaN_3) and was incubated with 300 mU/mL sialidase (*Vibrio cholera*)-agarose conjugate for 3 days at 32 °C. To monitor the reaction a small aliquot of the reaction was diluted with the appropriate buffer and an IEF gel performed according to Invitrogen procedures (Figure 157). The mixture was centrifuged at 3,500 rpm and the supernatant was collected. The resin was washed three times (3×2 mL) with the above buffer solution (pH 7.4, 0.05 M Tris, 0.15 M NaCl, 0.05% NaN_3) and the combined washes were concentrated in a Centricon-Plus-20. The remaining

solution was buffer exchanged with 0.05 M Tris (pH 7.4), 0.15 M NaCl, 0.05% NaN_3 to a final volume of 14.4 mL.

Preparation of Factor VIIa-SA-PEG (1 kDa and 10 kDa). The desialylation rFactor VIIa solution was split into two equal 7.2 ml samples. To each sample was added either CMP-SA-5-PEG(1 kDa) (7.4 mg) or CMP-SA-5-PEG(10 kDa) (7.4 mg). ST3Gal3 (1.58U) was added to both tubes and the reaction mixtures were incubated at 32°C for 96 hrs. The reaction was monitored by SDS-PAGE gel using reagents and conditions described by Invitrogen. When the reaction was complete, the reaction mixture was purified using a Toso Haas TSK-Gel-3000 preparative column using PBS buffer (pH 7.1) and collecting fractions based on UV absorption. The combined fractions containing the product were concentrated at 4°C in Centricon-Plus-20 centrifugal filters (Millipore, Bedford, MA) and the concentrated solution reformulated to yield 1.97 mg (bicinchoninic acid protein assay, BCA assay, Sigma-Aldrich, St. Louis MO) of Factor VIIa-PEG. The product of the reaction was analyzed using SDS-PAGE and IEF analysis according to the procedures and reagents supplied by Invitrogen. Samples were dialyzed against water and analyzed by MALDI-TOF. Figure 158 shows the MALDI results for native Factor VIIa. Figure 159 contains the MALDI results for Factor VIIa PEGylated with 1 kDa PEG where peak of Factor VIIa PEGylated with 1KDa PEG is evident. Figure 160 contains the MALDI results for Factor VIIa PEGylated with 10 kDa PEG where a peak for Factor VIIa PEGylated with 10 kDa PEG is evident. Figure 161 depicts the SDS-PAGE analysis of all of the reaction products, where a band for Factor VIIa-SA-PEG (10 kDa) is evident.

Follicle Stimulating Hormone (FSH)

23. GlycoPEGylation of human pituitary-derived FSH

This example illustrates the assembly of a conjugate of the invention. Follicle Stimulating Hormone (FSH) is desialylated and then conjugated with CMP-(sialic acid)-PEG.

Desialylation of Follicle Stimulating Hormone. Follicle Stimulating Hormone (FSH) (Human Pituitary, Calbiochem Cat No. 869001), 1 mg, was dissolved in 500 µL 50 mM Tris-HCl pH 7.4, 0.15 M NaCl, 5 mM $CaCl_2$. This solution, 375 µL, was transferred to a small plastic tube and to it was added 263 mU Neuraminidase II (*Vibrio cholerae*). The reaction mixture was shaken gently for 15 hours at 32 °C. The reaction mixture was added to

N-(*p*-aminophenyl)oxamic acid-agarose conjugate, 600 μL, pre-equilibrated with 50 mM Tris-HCl pH 7.4, 150 mM NaCl and 0.05% NaN_3 and gently rotated 6.5 hours at 4 °C. The suspension was centrifuged for 2 minutes at 14,000 rpm and the supernatant was collected. The beads were washed 5 times with 0.5 mL of the buffer and all supernatants were pooled. The enzyme solution was dialyzed (7000 MWCO) for 15 hours at 4 °C with 2 L of a solution containing 50 mM Tris –HCl pH 7.4, 1 M NaCl, 0.05% NaN_3, and then twice for 4 hours at 4 °C into 50 mM Tris –HCl pH 7.4, 1 M NaCl, 0.05% NaN_3. The solution was concentrated to 2 μg/μL by Speed Vac and stored at –20 °C. Reaction samples were analyzed by IEF gels (pH 3-7) (Invitrogen) (Figure 162).

Preparation of human pituitary-derived SA-FSH and PEG-SA-Follicle Stimulating Hormone. Desialylated FSH (100 μg, 50 μL) and CMP-sialic acid or CMP-SA-PEG (1 kDa or 10 kDa) (0.05 umol) were dissolved in 13.5 μL H_2O (adjusted to pH 8 with NaOH) in 0.5 mL plastic tubes. The tubes were vortexed briefly and 40 mU ST3Gal3 (36.5 μL) was added (total volume 100 μL). The tubes were vortexed again and shaken gently for 24 hours at 32 °C. The reactions were stopped by freezing at –80 °C. Reaction samples of 15 μg were analyzed by SDS-PAGE (Figure 163), IEF gels (Figure 164) and MALDI-TOF. Native FSH was also analyzed by SDS-PAGE (Figure 165)

Analysis of SDS PAGE and IEF Gels of Reaction Products. Novex Tris-Glycine 8-16% 1 mm gels for SDS PAGE analysis were purchased from Invitrogen. 7.5 μL (15 μg) of FSH reaction samples were diluted with 5 μL of 50 mM Tris-HCl, pH 7.4, 150 mM NaCl, 0.05% NaN_3 buffer, mixed with 15 μL sample loading buffer and 1 μL 9 M β-mercaptoethanol and heated for 6 minutes at 85 °C. Gels were run as directed by Invitrogen and stained with Colloidal Blue Stain (Invitrogen).

FSH samples (15 μg) were diluted with 5 μL Tris buffer and mixed with 15 μL sample loading buffer (Figure 162). The samples were then applied to Isoelectric Focusing Gels (pH 3-7) (Invitrogen) (Figure 165). Gels were run and fixed as directed by Invitrogen and then stained with Colloidal Blue Stain.

24. GlycoPEGylation of recombinant FSH produced recombinantly in CHO cells

This example illustrates the assembly of a conjugate of the invention. Desialylated FSH was conjugated with CMP-(sialic acid)-PEG.

Preparation of recombinant Asialo-Follicle Stimulation Hormone. Recombinant Follicle Stimulation Hormone (rFSH) produced from CHO was used in these studies. The 7,500 IU of rFSH was dissolved in 8 mL of water. The FSH solution was dialyzed in 50 mM Tris-HCl pH 7.4, 0.15 M NaCl, 5 mM $CaCl_2$ and concentrated to 500 μL in a Centricon Plus 20 centrifugal filter. A portion of this solution (400 μL) (~ 0.8 mg FSH) was transferred to a small plastic tube and to it was added 275 mU Neuraminidase II (*Vibrio cholerae*). The reaction mixture was mixed for 16 hours at 32 °C. The reaction mixture was added to prewashed N-(*p*-aminophenyl)oxamic acid-agarose conjugate (800 μL) and gently rotated for 24 hours at 4 °C. The mixture was centrifuged at 10,000 rpm and the supernatant was collected. The beads were washed 3 times with 0.6 mL Tris-EDTA buffer, once with 0.4 mL Tris-EDTA buffer and once with 0.2 mL of the Tris-EDTA buffer and all supernatants were pooled. The supernatant was dialyzed at 4 °C against 2 L of 50 mM Tris –HCl pH 7.4, 1 M NaCl, 0.05% NaN_3 and then twice more against 50 mM Tris –HCl pH 7.4, 1 M NaCl, 0.05% NaN_3. The dialyzed solution was then concentrated to 420 μL in a Centricon Plus 20 centrifugal filter and stored at –20 °C.

Native and desialylated rFSH samples were analyzed by SDS-PAGE and IEF (Figure 166). Novex Tris-Glycine 8-16% 1 mm gels were purchased from Invitrogen. Samples (7.5 μL, 15 μg) samples were diluted with 5 μL of 50 mM Tris-HCl, pH 7.4, 150 mM NaCl, 0.05% NaN_3 buffer, mixed with 15 μL sample loading buffer and 1 μL 9 M β-mercaptoethanol and heated for 6 minutes at 85 °C. Gels were run as directed by Invitrogen and stained with Colloidal Blue Stain (Invitrogen). Isoelectric Focusing Gels (pH 3-7) were purchased from Invitrogen. Samples (7.5 μL, 15 μg) were diluted with 5 μL Tris buffer and mixed with 15 μL sample loading buffer. Gels were loaded, run and fixed as directed by Invitrogen. Gels were stained with Colloidal Blue Stain. Samples of native and desialylated FSH were also dialyzed against water and analyzed by MALDI-TOF.

Sialyl-PEGylation of recombinant Follicle Stimulation Hormone. Desialylated FSH (100 μg, 54 μL) and CMP-SA-PEG (1 kDa or 10 kDa) (0.05 μmol) were dissolved in 28

μL 50 mM Tris-HCl, 0.15 M NaCl, 0.05% NaN_3, pH 7.2 in 0.5 mL plastic tubes. The tubes were vortexed briefly and 20 mU of ST3Gal3 was added (total volume 100 μL). The tubes were vortexed again, mixed gently for 24 hours at 32 °C and the reactions stopped by freezing at –80 °C. Samples of this reaction were analyzed as described above by SDS-PAGE gels (Figure 167), IEF gels (Figure 168) and MALDI-TOF MS.

MALDI was also performed on the PEGylated rFSH. During ionization, SA-PEG is eliminated from the N-glycan structure of the glycoprotein. Native FSH gave a peak at 13928; AS-rFSH (13282); resialylated r-FSH (13332); PEG1000-rFSH (13515; 14960 (1); 16455 (2); 17796 (3); 19321 (4)); and PEG 10000 (23560 (1); 34790 (2); 45670 (3); and 56760 (4)).

25. Pharmacokinetic Study of GlycoPEGylated FSH

This example sets forth the *in vivo* testing of the pharmacokinetic properties glycoPEGylated Follicle Stimulating Hormone (FSH) prepared according to the methods of the invention as compared to non-PEGylated FSH.

FSH, FSH-SA-PEG (1 kDa) and FSH-SA-PEG (10 kDa) were radioiodinated using standard conditions (Amersham Biosciences, Arlington Heights, IL) and formulated in phosphate buffered saline containing 0.1% BSA. After dilution in phosphate buffer to the appropriate concentration, each of the test FSH proteins (0.4 μg, each) was injected intraveneously into female Sprague Dawley rats (250-300 g body weight) and blood drawn at time points from 0 to 80 hours. Radioactivity in blood samples was analyzed using a gamma counter and the pharmacokinetics analyzed using standard methods (Figure 169). FSH was cleared from the blood much more quickly than FSH-PEG(1 kDa), which in turn was clear somewhat more quickly than FSH-PEG(10 kDa).

26. Sertoli Cell Bioassay for *In Vitro* Activity of GlycoPEGylated FSH

This example sets forth a bioassay for follicle stimulating hormone (FSH) activity based on cultured Sertoli cells. This assay is useful to determine the bioactivity of FSH after glycan remodeling, including glycoconjugation.

This bioassay is based on the dose-response relationship that exists between the amount of estradiol produced when FSH, but not lutenizing hormone (LH), is added to

cultured Sertoli cells obtained from immature old rats. Exogenous testosterone is converted to 17β-estradiol in the presence of FSH.

Seven to 10 days old Sprague-Dawley rats were used to obtain Sertoli cells. After sacrifice, testes were decapsulated and tissue was dispersed by incubation in collagenase (1 mg/ml), trypsin (1mg/ml), hyaluronidase (1 mg/ml) and DNases (5 μg/ml) for 5 to 10 min. The tubule fragments settled to the bottom of the flask and were washed in PBS (1x). The tubule fragments were reincubated for 20 min with a media containing the same enzymes: collagenase (1 mg/ml), trypsin (1mg/ml), hyaluronidase (1 mg/ml) and DNases (5 μg/ml).

The tubule fragments were homogenized and plated into a 24 well plate in a serum free media. 5 x 10^5 cells were dispersed per well. After 48h incubation at 37° C and 5% CO_2, fresh media was added to the cells. Composition of the serum free media: DMEM (1 vol), Ham's F10 nutrient mixture (1 vol), insulin 1 μg/ml, Transferrin 5 μg/ml, EGF 10 ng/ml, T4 20 pg/ml, Hydrocortisone 10^{-8} M, Retinoic acid 10^{-6} M.

The stimulation experiment consists of a 24 hour incubation with standard FSH or samples at 37°C and 5% CO_2. The mean intra-assay coefficient of variation is 9% and the mean inter-assay coefficient of variation is 11%.

The 17B-estradiol Elisa Kit DE2000 (R&D Systems, Minneapolis, MN) was used to quantify the level of estradiol after incubation with FSH, FSH-SA-PEG (1 kDa) and FSH-SA-PEG (10 kDa).

The procedure was as follows: 100 μl of Estradiol Standard (provided with kit and prepared as per instructions with kit) or sample was pipetted into wells of 17B-estradiol Elisa plate(s); 50 μl of 17B-estradiol Conjugate (provided with kit, prepared as per instructions with kit) was added to each well; 50 μl of 17B-estradiol antibody solution (provided with kit and prepared as per instructions with kit) was added to each well; plates were incubated for 2 hour at room temperature at 200 rpm; the liquid was aspirated from each well; the wells were washed 4 times using the washing solution; all the liquid was removed from the wells; 200 μl of pNPP Substrate (provided with kit and prepared as per instructions with kit) was added to all wells and incubated for 45 min; 50 μl of Stop solution (provided with kit and prepared as per instructions with kit) was added and the plates were read it at 405 nm (Figure 170). While FSH-PEG(10 kDa) exhibited a modest stimulation of Sertoli cells, at 1 μg/ml, FSH-PEG(1 kDa) stimulated Sertoli cells up to 50% more than unPEGylated FSH.

27. Steelman-Pohley Bioassay of *In Vivo* Activity of GlycoPEGylated FSH

In this example, the Steelman-Pohley bioassay (Steelman and Pohley, 1953, Endocrinology 53:604-615) was used to determine the *in vivo* activity of glycoPEGylated FSH. The Steelman-Pohley assay uses the change in ovary weight of a rat to measure the *in vivo* activity of FSH that is coinjected with human chorionic gonadotropin.

The Steelman-Pohley bioassay was performed according to the protocol described in Christin-Maitre et al. (2000, Methods 21:51-57). Seventy female Sprague-Dawley Rats (Charles River Laboratories, Wilmington, MA), aged 21 to 22 days, were housed in the testing facility for at least 5 days before the beginning the assay procedure. Throughout the procedure, the animal room was climate controlled at 18 to 26°C, 30 to 70% relative humidity, and 12 hr. artificial light/12 hr. dark. All animals were fed Certified Rodent Chow (Harlan Teklad, Madison WI) or the equivalent, and water, both *ad libitum*. Animal procedures were performed at Calvert Preclinical Services, Inc. (Olyphant, PA).

Recombinant FSH was expressed in CHO cells, purified by standard techniques and glycoPEGylated with PEG (1 kDa). The rats were divided into seven test groups, with ten animals per group. On days –1 and 0, animals of all groups were subcutaneously injected with 20 I.U. of human chorionic gonadotropin (HCG) in 0.5 ml of 0.9 % NaCl. On days 1, 2 and 3, the control animals were subcutaneously injected with a dose of 0.5 ml containing 20 I.U. HCG in 0.9% NaCl, while in the other groups, the HCG dose was augmented with either rFSH or rFSH-SA-PEG (1 kDa) at either 0.14 μg, 0.4 μg or 1.2 μg per dose. On day 4, the animals were euthanized by CO_2 inhalation. The ovaries were removed, trimmed and weighted. The average ovary weight was determined for each group.

Figure 171 presents the average ovary weight of the test groups on day 4. The groups receiving HCG alone (control) or the low dose (0.14 μg) of either rFSH or rFSH-SA-PEG (1 kDa) had ovary weights that were roughly equivalent. The groups receiving the medium (0.4 μg) or high (1.2 μg) doses of rFSH or rFSH-SA-PEG (1 kDa) had ovary weights roughly twice that of the control group. At the medium dose (0.4 μg), the glycoPEGylated rFSH had roughly the same *in vivo* activity (as determined by ovary weight) as the unPEGylated rFSH.

At the high dose (1.2 μg), the glycoPEGylated rFSH had somewhat higher *in vivo* activity than the unPEGylated rFSH.

G-CSF

28. GlycoPEGylation of G-CSF produced in CHO cells

Preparation of Asialo-Granulocyte-Colony Stimulation Factor (G-CSF). G-CSF produced in CHO cells is dissolved at 2.5 mg/mL in 50 mM Tris 50 mM Tris-HCl pH 7.4, 0.15 M NaCl, 5 mM $CaCl_2$ and concentrated to 500 μL in a Centricon Plus 20 centrifugal filter. The solution is incubated with 300 mU/mL Neuraminidase II (*Vibrio cholerae*) for 16 hours at 32 °C. To monitor the reaction a small aliquot of the reaction is diluted with the appropriate buffer and a IEF gel performed. The reaction mixture is then added to prewashed N-(*p*-aminophenyl)oxamic acid-agarose conjugate (800 μL/mL reaction volume) and the washed beads gently rotated for 24 hours at 4 °C. The mixture is centrifuged at 10,000 rpm and the supernatant was collected. The beads are washed 3 times with Tris-EDTA buffer, once with 0.4 mL Tris-EDTA buffer and once with 0.2 mL of the Tris-EDTA buffer and all supernatants are pooled. The supernatant is dialyzed at 4 °C against 50 mM Tris –HCl pH 7.4, 1 M NaCl, 0.05% NaN_3 and then twice more against 50 mM Tris –HCl pH 7.4, 1 M NaCl, 0.05% NaN_3. The dialyzed solution is then concentrated using a Centricon Plus 20 centrifugal filter and stored at –20 °C. The conditions for the IEF gel were run according to the procedures and reagents provided by Invitrogen. Samples of native and desialylated G-CSF are dialyzed against water and analyzed by MALDI-TOF MS.

Preparation of G-CSF-(alpha2,3)-Sialyl-PEG. Desialylated G-CSF was dissolved at 2.5 mg/mL in 50 mM Tris-HCl, 0.15 M NaCl, 0.05% NaN_3, pH 7.2. The solution is incubated with 1 mM CMP-sialic acid-PEG and 0.1 U/mL of ST3Gal1 at 32°C for 2 days. To monitor the incorporation of sialic acid-PEG, a small aliquot of the reaction had CMP-SA-PEG-fluorescent ligand added; the label incorporated into the peptide is separated from the free label by gel filtration on a Toso Haas G3000SW analytical column using PBS buffer (pH 7.1). The fluorescent label incorporation into the peptide is quantitated using an in-line fluorescent detector. After 2 days, the reaction mixture is purified using a Toso Haas G3000SW preparative column using PBS buffer (pH 7.1) and collecting fractions based on UV absorption. The product of the reaction is analyzed using SDS-PAGE and IEF analysis

according to the procedures and reagents supplied by Invitrogen. Samples of native and PEGylated G-CSF are dialyzed against water and analyzed by MALDI-TOF MS.

Preparation of G-CSF-(alpha2,8)-Sialyl-PEG. G-CSF produced in CHO cells, which contains an alpha2,3-sialylated O-linked glycan, is dissolved at 2.5 mg/mL in 50 mM Tris-HCl, 0.15 M NaCl, 0.05% NaN_3, pH 7.2. The solution is incubated with 1 mM CMP-sialic acid-PEG and 0.1 U/mL of CST-II at 32°C for 2 days. To monitor the incorporation of sialic acid-PEG, a small aliquot of the reaction has CMP-SA-PEG-fluorescent ligand added; the label incorporated into the peptide is separated from the free label by gel filtration on a Toso Haas G3000SW analytical column using PBS buffer (pH 7.1). The fluorescent label incorporation into the peptide is quantitated using an in-line fluorescent detector. After 2 days, the reaction mixture is purified using a Toso Haas G3000SW preparative column using PBS buffer (pH 7.1) and collecting fractions based on UV absorption. The product of the reaction is analyzed using SDS-PAGE and IEF analysis according to the procedures and reagents supplied by Invitrogen. Samples of native and PEGylated G-CSF are dialyzed against water and analyzed by MALDI-TOF MS.

Preparation of G-CSF-(alpha2,6)-Sialyl-PEG. G-CSF, containing only O-linked GalNAc, is dissolved at 2.5 mg/mL in 50 mM Tris-HCl, 0.15 M NaCl, 0.05% NaN_3, pH 7.2. The solution is incubated with 1 mM CMP-sialic acid-PEG and 0.1 U/mL of ST6GalNAcI or II at 32°C for 2 days. To monitor the incorporation of sialic acid-PEG, a small aliquot of the reaction has CMP-SA-PEG-fluorescent ligand added; the label incorporated into the peptide is separated from the free label by gel filtration on a Toso Haas G3000SW analytical column using PBS buffer (pH 7.1). The fluorescent label incorporation into the peptide is quantitated using an in-line fluorescent detector. After 2 days, the reaction mixture is purified using a Toso Haas G3000SW preparative column using PBS buffer (pH 7.1) and collecting fractions based on UV absorption. The product of the reaction is analyzed using SDS-PAGE and IEF analysis according to the procedures and reagents supplied by Invitrogen. Samples of native and PEGylated G-CSF are dialyzed against water and analyzed by MALDI-TOF MS.

G-CSF produced in CHO cells was treated with Arthrobacter sialidase and was then purified by size exclusion on Superdex75 and was treated with ST3Gal1 or ST3 Gal2 and then with CMP-SA-PEG 20Kda. The resulting molecule was purified by ion exchange and

gel filtration and analysis by SDS PAGE demonstrated that the PEGylation was complete. This is the first demonstration of glycoPEGylation of an O-linked glycan.

Glucocerebrosidase

29. Glucocerebrosidase-mannose-6-phosphate produced in CHO cells

This example sets forth the procedure to glycoconjugate mannose-6-phosphate to a peptide produced in CHO cells such as glucocerebrosidase.

Preparation of asialo-glucoceramidase. Glucocerebrosidase produced in CHO cells is dissolved at 2.5 mg/mL in 50 mM Tris 50 mM Tris-HCl pH 7.4, 0.15 M NaCl, and is incubated with 300 mU/mL sialidase-agarose conjugate for 16 hours at 32 °C. To monitor the reaction a small aliquot of the reaction is diluted with the appropriate buffer and a IEF gel and SDS-PAGE performed according to Invitrogen procedures. The mixture is centrifuged at 10,000 rpm and the supernatant is collected. The beads are washed 3 times with Tris-EDTA buffer, once with 0.4 mL Tris-EDTA buffer, and once with 0.2 mL of the Tris-EDTA buffer. All supernatants are pooled. The supernatant is dialyzed at 4 °C against 50 mM Tris–HCl pH 7.4, 1 M NaCl, 0.05% NaN_3 and then twice more against 50 mM Tris–HCl pH 7.4, 1 M NaCl, 0.05% NaN_3. The dialyzed solution is then concentrated using a Centricon Plus 20 centrifugal filter. The product of the reaction is analyzed using SDS-PAGE and IEF analysis according to the procedures and reagents supplied by Invitrogen. Samples are dialyzed against water and analyzed by MALDI-TOF MS.

Preparation of Glucocerebrosidase-SA-linker-Mannose-6-phosphate (procedure 1). Asialo-glucocerebrosidasefrom above is dissolved at 2.5 mg/mL in 50 mM Tris-HCl, 0.15 M NaCl, 0.05% NaN_3, pH 7.2. The solution is incubated with 1 mM CMP-sialic acid-linker-Man-6-phosphate and 0.1 U/mL of ST3Gal3 at 32°C for 2 days. To monitor the incorporation of sialic acid-linker-Man-6-phosphate, a small aliquot of the reaction had CMP-SA-PEG-fluorescent ligand added; the label incorporated into the peptide is separated from the free label by gel filtration on a Toso Haas TSK-Gel-3000 analytical column using PBS buffer (pH 7.1). The fluorescent label incorporation into the peptide is quantitated using an in-line fluorescent detector. When the reaction is complete, the reaction mixture is purified using a Toso Haas TSK-Gel-3000 preparative column using PBS buffer (pH 7.1) and collecting fractions based on UV absorption. The product of the reaction is analyzed using

SDS-PAGE and IEF analysis according to the procedures and reagents supplied by Invitrogen. Samples are dialyzed against water and analyzed by MALDI-TOF MS.

Preparation of Glucocerebrosidase-SA-linker-Mannose-6-phosphate (procedure 2). Glucocerebrosidase, produced in CHO but incompletely sialylated, is dissolved at 2.5 mg/mL in 50 mM Tris-HCl, 0.15 M NaCl, 0.05% NaN_3, pH 7.2. The solution is incubated with 1 mM CMP-sialic acid-linker-Man-6-phosphate and 0.1 U/mL of ST3Gal3 at 32°C for 2 days. To monitor the incorporation of sialic acid-linker-Man-6-phosphate, a small aliquot of the reaction had CMP-SA-PEG-fluorescent ligand added; the label incorporated into the peptide is separated from the free label by gel filtration on a Toso Haas TSK-Gel-3000 analytical column using PBS buffer (pH 7.1). The fluorescent label incorporation into the peptide is quantitated using an in-line fluorescent detector. When the reaction is complete, the reaction mixture is purified using a Toso Haas TSK-Gel-3000 preparative column using PBS buffer (pH 7.1) and collecting fractions based on UV absorption. The product of the reaction is analyzed using SDS-PAGE and IEF analysis according to the procedures and reagents supplied by Invitrogen. Samples are dialyzed against water and analyzed by MALDI-TOF MS.

30. Glucocerebrosidase-transferrin

This example sets forth the procedures for the glycoconjugation of proteins, and in particular, transferrin is glycoconjugated to glucocerebrosidase. The GlcNAc-ASN structures are created on glucoceraminidase, and Transferrin-SA-Linker-Gal-UDP is conjugated to GNDF GlcNAc-ASN structures using galactosyltransferase.

Preparation of GlcNAc-glucocerebrosidase (Cerezyme™). Cerezyme™ (glucocerebrosidase) produced in CHO cells is dissolved at 2.5 mg/mL in 50 mM Tris 50 mM Tris-HCl pH 7.4, 0.15 M NaCl, and is incubated with 300 mU/mL Endo-H-agarose conjugate for 16 hours at 32 °C. To monitor the reaction a small aliquot of the reaction is diluted with the appropriate buffer and a IEF gel and SDS-PAGE performed according to Invitrogen procedures. The mixture is centrifuged at 10,000 rpm and the supernatant is collected. The beads are washed 3 times with Tris-EDTA buffer, once with 0.4 mL Tris-EDTA buffer and once with 0.2 mL of the Tris-EDTA buffer and all supernatants are pooled. The supernatant is dialyzed at 4 °C against 50 mM Tris –HCl pH 7.4, 1 M NaCl, 0.05% NaN_3 and then twice

more against 50 mM Tris –HCl pH 7.4, 1 M NaCl, 0.05% NaN_3. The dialyzed solution is then concentrated using a Centricon Plus 20 centrifugal filter. The product of the reaction is analyzed using SDS-PAGE and IEF analysis according to the procedures and reagents supplied by Invitrogen. Samples are dialyzed against water and analyzed by MALDI-TOF MS.

Preparation of Transferrin-SA-Linker-Gal-glucocerebrosidase. Transferrin-SA-Linker-Gal-UDP from above is dissolved at 2.5 mg/mL in 50 mM Tris-HCl, 0.15 M NaCl, 5 mM $MnCl_2$, 0.05% NaN_3, pH 7.2. The solution is incubated with 2.5 mg/mL GlcNAc-glucocerebrosidaseand 0.1 U/mL of galactosyltransferase at 32°C for 2 days. To monitor the incorporation of glucocerebrosidase, the peptide is separated by gel filtration on a Toso Haas G3000SW analytical column using PBS buffer (pH 7.1) and the product detected by UV absorption. The reaction mixture is then purified using a Toso Haas G3000SW preparative column using PBS buffer (pH 7.1) collecting fractions based on UV absorption. The product of the reaction is analyzed using SDS-PAGE and IEF analysis according to the procedures and reagents supplied by Invitrogen. Samples are dialyzed against water and analyzed by MALDI-TOF MS.

GM-CSF

31. Generation and PEGylation of GlcNAc-ASN Structures: GM-CSF produced in *Saccharomyces*

This example sets forth the preparation of Tissue-type Activator with PEGylated GlcNAc-Asn structures.

Recombinant GM-CSF expressed in yeast is expected to contain 2 N-linked and 2 O-linked glycans. The N-linked glycans should be of the branched mannan type. This recombinant glycoprotein is treated with an endoglycosidase from the group consisting of endoglycosidase H, endoglycosidase-F1, endoglycosidase-F2, endoglycosidase-F3, endoglycosidase-M either alone or in combination with mannosidases I, II and III to generate GlcNAc nubs on the asparagine (Asn) residues on the peptide/protein backbone.

The GlcNAc-Asn structures on the peptide/protein backbone is then be modified with galactose or galactose-PEG using UDP-galactose or UDP-galactose-6-PEG, respectively, and a galactosyltransferase such as GalT1. In one case the galactose-PEG is the terminal residue.

In the second case the galactose is further modified with SA-PEG using a CMP-SA-PEG donor and a sialyltransferase such as ST3GalIII. In another embodiment the GlcNAc-Asn structures on the peptide/protein backbone can be galactosylated and sialylated as described above, and then further sialylated using CMP-SA-PEG and an α2,8-sialyltranferase such as the enzyme encoded by the *Campylobacter jejuni* cst-II gene.

Herceptin™

32. Glycoconjugation of mithramycin to Herceptin™

This example sets forth the procedures to glycoconjugate a small molecule, such as mithramycin to Fc region glycans of an antibody molecule produced in mammalian cells. Here, the antibody Herceptin™ is used, but one of skill in the art will appreciate that the method can be used with many other antibodies.

Preparation of Herceptin™-Gal-linker-mithramycin. Herceptin™ is dissolved at 2.5 mg/mL in 50 mM Tris-HCl, 0.15 M NaCl, 5 mM $MnCl_2$, 0.05% NaN_3, pH 7.2. The solution is incubated with 1 mM UDP-galactose-linker-mithramycin and 0.1 U/mL of galactosyltransferase at 32°C for 2 days to introduce the mithramycin in the Fc region glycans. To monitor the incorporation of galactose, a small aliquot of the reaction has ^{14}C-galactose-UDP ligand added; the label incorporated into the peptide is separated from the free label by gel filtration on a Toso Haas G3000SW analytical column using PBS buffer (pH 7.1). The radioactive label incorporation into the peptide is quantitated using an in-line radiation detector.

When the reaction is complete, the reaction mixture is purified using a Toso Haas TSK-Gel-3000 preparative column using PBS buffer (pH 7.1) and collecting fractions based on UV absorption. The fractions containing product are combined, concentrated, buffer exchanged and then freeze-dried. The product of the reaction is analyzed using SDS-PAGE and IEF analysis according to the procedures and reagents supplied by Invitrogen. Samples are dialyzed against water and analyzed by MALDI-TOF MS.

Interferon α and Interferon β

33. GlycoPEGylation of Proteins expressed in Mammalian or Insect Systems: EPO, Interferon α and Interferon β

This example sets forth the preparation of PEGylated peptides that are expressed in mammalian and insect systems.

Preparation of acceptor from mammalian expression systems. The peptides to be glycoPEGylated using CMP-sialic acid PEG need to have glycans terminating in galactose. Most peptides from mammalian expression systems will have terminal sialic acid that first needs to be removed.

Sialidase digestion. The peptide is desialylated using a sialidase. A typical procedure involves incubating a 1 mg/mL solution of the peptide in Tris-buffered saline, pH 7.2, with 5 mM $CaCl_2$ added, with 0.2 U/mL immobilized sialidase from *Vibrio cholera* (Calbiochem) at 32°C for 24 hours. Microbial growth can be halted either by sterile filtration or the inclusion of 0.02% sodium azide. The resin is then removed by centrifugation or filtration, and then washed to recover entrapped peptide. At this point, EDTA may be added to the solution to inhibit any sialidase that has leached from the resin.

Preparation from insect expression systems. EPO, interferon-alpha, and interferon-beta may also be expressed in non-mammalian systems such as yeast, plants, or insect cells. The peptides to be glycoPEGylated using CMP-sialic acid PEG need to have glycans terminating in galactose. The majority of the N-glycans on peptides expressed in insect cells, for example, are the trimannosyl core. These glycans are first built out to glycans terminating in galactose before they are acceptors for sialyltransferase.

Building acceptor glycans from trimannosyl core. Peptide (1 mg/mL) in Tris-buffered saline, pH 7.2, containing 5 mM $MnCl_2$, 5 mM UDP-glcNAc, 0.05 U/mL GLCNACT I, 0.05 U/mL GLCNACT II, is incubated at 32°C for 24 hours or until the reaction is substantially complete. Microbial growth can be halted either by sterile filtration or the inclusion of 0.02% sodium azide. After buffer exchange to remove UDP and other small molecules, UDP-galactose and $MnCl_2$ are each added to 5 mM, galactosyltransferase is added to 0.05 U/mL, and is incubated at 32°C for 24H or until the reaction is substantially

complete. Microbial growth can be halted either by sterile filtration or the inclusion of 0.02% sodium azide. The peptides are then ready for glycoPEGylation.

Building O-linked glycans. A similar strategy may be employed for interferon alpha to produce enzymatically the desired O-glycan Gal-GalNAc. If necessary, GalNAc linked to serine or threonine can be added to the peptide using appropriate peptide GalNAc transferases (e.g. GalNAc T1, GalNAc T2, T3, T4, etc.) and UDP-GalNAc. Also, if needed, galactose can be added using galactosyltransferase and UDP-galactose.

GlycoPEGylation using sialyltransferase. The glycopeptides (1 mg/mL) bearing terminal galactose in Tris buffered saline + 0.02% sodium azide are incubated with CMP-SA-PEG (0.75 mM) and 0.4 U/mL sialyltransferase (ST3Gal3 or ST3Gal4 for N-glycans on EPO and interferon beta; ST3Gal4, or ST3Gal1 for O-glycans on interferon alpha) at 32°C for 24 hours. Other transferases that may work include the 2,6 sialyltransferase from *Photobacterium damsella*. The acceptor peptide concentration is most preferably in the range of 0.1 mg/mL up to the solubility limit of the peptide. The concentration of CMP-SA-PEG should be sufficient for there to be excess over the available sites, but not so high as to cause peptide solubility problems due to the PEG, and may range from 50 μM up to 5 mM, and the temperature may range from 2°C up to 40°C. The time required for complete reaction will depend on the temperature, the relative amounts of enzyme to acceptor substrate, the donor substrate concentration, and the pH.

34. GlycoPEGylation of Interferon α produced in CHO cells

Preparation of Asialo-Interferon α. Interferon alpha produced from CHO cells is dissolved at 2.5 mg/mL in 50 mM Tris 50 mM Tris-HCl pH 7.4, 0.15 M NaCl, 5 mM $CaCl_2$ and concentrated to 500 μL in a Centricon Plus 20 centrifugal filter. The solution is incubated with 300 mU/mL Neuraminidase II (*Vibrio cholerae*) for 16 hours at 32 °C. To monitor the reaction a small aliquot of the reaction is diluted with the appropriate buffer and a IEF gel performed. The reaction mixture is then added to prewashed N-(*p*-aminophenyl)oxamic acid-agarose conjugate (800 μL/mL reaction volume) and the washed beads gently rotated for 24 hours at 4 °C. The mixture is centrifuged at 10,000 rpm and the supernatant was collected. The beads are washed 3 times with Tris-EDTA buffer, once with 0.4 mL Tris-EDTA buffer and once with 0.2 mL of the Tris-EDTA buffer and all

supernatants were pooled. The supernatant is dialyzed at 4 °C against 50 mM Tris –HCl pH 7.4, 1 M NaCl, 0.05% NaN_3 and then twice more against 50 mM Tris –HCl pH 7.4, 1 M NaCl, 0.05% NaN_3. The dialyzed solution is then concentrated using a Centricon Plus 20 centrifugal filter and stored at –20 °C. The conditions for the IEF gel are run according to the procedures and reagents provided by Invitrogen. Samples of native and desialylated G-CSF are dialyzed against water and analyzed by MALDI-TOF MS.

Preparation of Interferon-alpha-(alpha2,3)-Sialyl-PEG. Desialylated interferon-alpha is dissolved at 2.5 mg/mL in 50 mM Tris-HCl, 0.15 M NaCl, 0.05% NaN_3, pH 7.2. The solution is incubated with 1 mM CMP-sialic acid-PEG and 0.1 U/mL of ST3Gal1 at 32°C for 2 days. To monitor the incorporation of sialic acid-PEG, a small aliquot of the reaction had CMP-SA-PEG-fluorescent ligand added; the label incorporated into the peptide is separated from the free label by gel filtration on a Toso Haas G3000SW analytical column using PBS buffer (pH 7.1). The fluorescent label incorporation into the peptide is quantitated using an in-line fluorescent detector. After 2 days, the reaction mixture is purified using a Toso Haas G3000SW preparative column using PBS buffer (pH 7.1) and collecting fractions based on UV absorption. The product of the reaction is analyzed using SDS-PAGE and IEF analysis according to the procedures and reagents supplied by Invitrogen. Samples of native and desialylated Interferon-alpha are dialyzed against water and analyzed by MALDI-TOF MS.

Preparation of Interferon-alpha-(alpha2,8)-Sialyl-PEG. Interferon-alpha produced in CHO, which contains an alpha2,3-sialylated O-linked glycan, is dissolved at 2.5 mg/mL in 50 mM Tris-HCl, 0.15 M NaCl, 0.05% NaN_3, pH 7.2. The solution is incubated with 1 mM CMP-sialic acid-PEG and 0.1 U/mL of CST-II at 32°C for 2 days. To monitor the incorporation of sialic acid-PEG, a small aliquot of the reaction has CMP-SA-PEG-fluorescent ligand added; the label incorporated into the peptide is separated from the free label by gel filtration on a Toso Haas G3000SW analytical column using PBS buffer (pH 7.1). The fluorescent label incorporation into the peptide is quantitated using an in-line fluorescent detector. After 2 days, the reaction mixture is purified using a Toso Haas G3000SW preparative column using PBS buffer (pH 7.1) and collecting fractions based on UV absorption. The product of the reaction is analyzed using SDS-PAGE and IEF analysis

according to the procedures and reagents supplied by Invitrogen. Samples of native and PEGylated interferon-alpha are dialyzed against water and analyzed by MALDI-TOF MS.

Preparation of Interferon-alpha-(alpha2,6)-Sialyl-PEG. Interferon-alpha, containing only O-linked GalNAc, was dissolved at 2.5 mg/mL in 50 mM Tris-HCl, 0.15 M NaCl, 0.05% NaN_3, pH 7.2. The solution is incubated with 1 mM CMP-sialic acid-PEG and 0.1 U/mL of ST6GalNAcI or II at 32°C for 2 days. To monitor the incorporation of sialic acid-PEG, a small aliquot of the reaction had CMP-SA-PEG-fluorescent ligand added; the label incorporated into the peptide is separated from the free label by gel filtration on a Toso Haas G3000SW analytical column using PBS buffer (pH 7.1). The fluorescent label incorporation into the peptide is quantitated using an in-line fluorescent detector. After 2 days, the reaction mixture is purified using a Toso Haas G3000SW preparative column using PBS buffer (pH 7.1) and collecting fractions based on UV absorption. The product of the reaction is analyzed using SDS-PAGE and IEF analysis according to the procedures and reagents supplied by Invitrogen. Samples of native and PEGylated interferon-alpha are dialyzed against water and analyzed by MALDI-TOF MS.

35. GlycoPEGylation of Interferon-β-1a with PEG (10 kDa) and PEG (20 kDa)

This example illustrates a procedure PEGylate Interferon-β with either PEG (10 kDa) or PEG (20 kDa).

Briefly, Interferon-β-1a (INF-β) was obtained from Biogen (Avonex™). The IFN-β was first purified by Superdex-75 chromatography. The IFN-β was then desialylated with *Vibrio cholerae* sialidase. The INF-β was then PEGylated with SA-PEG (10 kDa) or SA-PEG (20 kDa) and purified with Superdex-200 chromatography.

Superdex-75 chromatography purification. INF-β (150 μg) was applied to a Superdex-75 column (Amersham Biosciences, Arlington Heights, IL) and eluted with PBS with 0.5 M NaCl, 0.02 Tween-20, 20 mM histidine and 10% glycerol. The eluant was monitored for absorbance at 280 nm (Figure 172A and 172B) and fractions were collected. Peaks 4 and 5 were pooled, concentrated in an Amicon Ultra 15 spin filter (Millipore, Billerica, MA), and the buffer was exchanged to TBS with 5 mM $CaCl_2$, 0.02% Tween-20, 20 mM histidine and 10% glycerol.

Sialidase Reaction. The INF-β was then desialydated with *Vibrio cholera* salidase (70 mU/ml, CALBIOCHEM®, EMD Biosciences, Inc., San Diego, CA) on agarose in TBS with 5 mM $CaCl_2$, 0.02% Tween-20, 20 mM histidine and 10% glycerol. The reaction was carried out at 32°C for 18 hours. The INF-β was removed from the agarose with a 0.22 μm Spin-X™ filter (Corning Technology, Inc., Norcross, GA). Figure 173A depicts the MALDI analysis of glycans released from native INF-β. The native INF-β has many glycoforms containing terminal sialic acid moieties. Figure 173B depicts the MALDI analysis of glycans released from desialylated INF-β. The desialylated INF-β has primarily one glycoform which is bi-antennary with terminal galactose moieties.

Lectin Dot-Blot Analysis of Sialylation. Samples of the INF-β from the desialidase reaction were dot-blotted onto nitrocellulose and then blocked with Tris buffered saline (TBS: 0.05M Tris, 0.15M NaCl, pH 7.5) and DIG kit (glycan differentiation kit available from Roche #1 210 238) blocking buffer. Some of the blots were incubated with *Maackia amurensis* agglutinin (MAA) labeled with digoxogenin (DIG) (Roche Applied Science, Indianapolis, IL) to detect α2,3-sialylation of INF-β. These blots were washed with TBS then incubated with anti-digitonin antibody labeled with alkaline phosphatase, then washed again with TBS and developed withNBT/X-phosphate solution, wherein NBT is 4-nitro blue tetrazolium chloride and X-phosphate is 5-bromo-4-chloro—3indoyl phosphate. The left side of Figure 174 depicts the results of the MAA blot of INF-β after the desialylation reaction. The INF-β is partially disialylated, as indicated by the decrease in dot development as compared to native INF-β in the desialylated samples.

Other blots were incubated with *Erthrina cristagalli* lectin (ECL) labeled with biotin (Vector Laboratories, Burlingame, CA) to detect exposed galactose residues on INF-β. After incubation with 2.5 μg/ml ECL, the blots were washed in TBS and incubated with streptavidin labeled with alkaline phosphatase. The blots were then washed again and developed. The right side of Figure 174 depicts the ECL blot after development. The increased intensity of the dot of desialylated INF-β as compared to the native INF-β indicate more exposed galactose moieties and therefore extensive desialylation.

PEGylation of Desialylated INF-β with SA-PEG (10 kDa). Desialylated INF-β (0.05 mg/ml) was PEGylated with ST3Gal3 (50 mU/ml) and CMP-SA-PEG (10 kDa) (250

μM) in an appropriate buffer of TBS + 5 mM $CaCl_2$, 0.02% Tween 20, 20 mM histidine, 10% glycerol for 50 hours at 32°C. Figure 175 depicts the SDS-PAGE analysis of the reaction products showing PEGylated INF-β at approximately 98 kDa.

PEGylation of Desialylated INF-β with SA-PEG (20 kDa). Desialylated INF-β (0.5 mg/ml) was PEGylated with ST3Gal3 (170 mU/ml) and CMP-SA-PEG (20 kDa) in an appropriate buffer of TBS + 5 mM $CaCl_2$, 0.02% Tween 20, 20 mM histidine, 10% glycerol for 50 hours at 32°C. Figure 176 depicts the SDS-PAGE analysis the products of the PEGylation reaction. The PEGylated INF-β has many higher molecular weight bands not found in the unmodified INF-β indicating extensive PEGylation.

Superdex-200 Purification of INF-β PEGylated with PEG (10 kDa). The products of the PEGylation reaction were separated on a Superdex-200 column (Amersham Biosciences, Arlington Heights, IL) in PBS with 0.5 NaCl, 0.02 Tween-20, 20 mM histidine and 10% glycerol at 1ml/min and 30 cm/hr flow. The eluant was monitored for absorbance at 280 nm (Figure 177) and fractions were collected. Peaks 3 and 4 were pooled and concentrated in an Amicon Ultra 15 spin filter.

Bioassay of INF-β PEGylated with PEG (10 kDa).

The test is inhibition of the proliferation of the lung carcinoma cell line, A549. The A549 cell line are lung carcinoma adherent cells growing in RPMI + 10% FBS at 37°C 5% CO_2. They can be obtained from ATCC # CCL-185. Wash the cells with 10 ml of PBS and remove the PBS. Add 5 ml of trypsin, incubate for 5 minutes at room temperature or 2 minutes at 37°C. When the cells are detached resuspend into 25 ml of media and count the cells. Dilute the cells at a concentration of 10000 cells/ml and add 200 ul / well (96 wells plate). Incubate for 4 hours at 37°C 5% CO_2. Prepare 1 ml of IFN B at a concentration of 0.1 ug/ml. Filter it under the hood with a 0.2 um filter. Add 100 ul per well (8 replicates = 1 lane). Incubate for 3 days (do not let the cells go to confluence). Remove 200 ul of media (only 100ul per well left). Add 25 μl of MTT (Sigma) (5 mg/ml filtered 0.22μm). Incubate for 4 hours at 37°C and 5% CO_2. Aspirate the media gently and add 100 μl of a mixture of isopropanol (100 ml and 6N HCl. Aspirate up and down to homogenize the crystal violet. Read OD 570nm (remove the background at 630 or 690 nm).

Figure 178 depicts the results of the bioassay of the peaks containing INF-β PEGylated with PEG (10 kDa) as eluted from the Superdex-200 column.

Superdex-200 Purification of INF-β PEGylated with PEG (20 kDa). The products of the PEG (20 kDa) PEGylation reaction were separated on a Superdex-200 column (Amersham Biosciences, Arlington Heights, IL) in PBS with 0.5 NaCl, 0.02 Tween-20, 20 mM histidine and 10% glycerol at 1 ml/min flow. The eluant was monitored for absorbance at 280 nm (Figure 179) and fractions were collected. Peak 3 contained most of the INF-β PEGylated with PEG (20 kDa).

Endotoxin test of INF-β PEGylated with PEG (20 kDa).

Limulus Lysate Test was performed, BioWhittaker # 50-647U

Table 24. Results of the endotoxin test of INF-β PEGylated with PEG (20 kDa).

	Concentration		
INF-β with PEG (20 kDa)	10 EU/ml	0.06 mg/ml	0.16 EU/μg
INF-β with PEG (20 kDa)	1 EU/ml	0.07 mg/ml	0.014 EU/μg
Native INF-β	40 EU/ml	0.1 mg/ml	0.4 EU/μg

Remicade™

36. GlycoPEGylation of Remicade™ antibody

This example sets forth the procedure to glycoPEGylate a recombinant antibody molecule by introducing PEG molecules to the Fc region glycans. Here Remicade™, a TNF-R:IgG Fc region fusion protein, is the exemplary peptide.

Preparation of Remicade™-Gal-PEG (10 kDa). Remicade™ is dissolved at 2.5 mg/mL in 50 mM Tris-HCl, 0.15 M NaCl, 5 mM $MnCl_2$, 0.05% NaN_3, pH 7.2. The solution is incubated with 1 mM UDP-galactose-PEG (10 kDa) and 0.1 U/mL of galactosyltransferase at 32°C for 2 days to introduce the PEG in the Fc region glycans. To monitor the incorporation of galactose, a small aliquot of the reaction has ^{14}C-galactose-UDP ligand added; the label incorporated into the peptide is separated from the free label by gel filtration on a Toso Haas G3000SW analytical column using PBS buffer (pH 7.1). The radioactive label incorporation into the peptide is quantitated using an in-line radiation detector.

When the reaction is complete, the reaction mixture is purified using a Toso Haas TSK-Gel-3000 preparative column using PBS buffer (pH 7.1) and collecting fractions based on UV absorption. The fractions containing product are combined, concentrated, buffer exchanged and then freeze-dried. The product of the reaction is analyzed using SDS-PAGE and IEF analysis according to the procedures and reagents supplied by Invitrogen. Samples are dialyzed against water and analyzed by MALDI-TOF MS.

Rituxan™

37. Glycoconjugation of geldanamycin to Rituxan™

This example sets forth the glycoconjugation of a small molecule, such as geldanamycin, to the Fc region glycans of an antibody produced in CHO cells, such as Rituxan™. Here, the antibody Rituxan™ is used, but one of skill in the art will appreciate that the method can be used with many other antibodies.

Preparation of Rituxan™-Gal-linker-geldanamycin. Rituxan™ is dissolved at 2.5 mg/mL in 50 mM Tris-HCl, 0.15 M NaCl, 5 mM $MnCl_2$, 0.05% NaN_3, pH 7.2. The solution is incubated with 1 mM UDP-galactose-linker-geldanamycin and 0.1 U/mL of galactosyltransferase at 32°C for 2 days to introduce the geldanamycin in the Fc region glycans. To monitor the incorporation of galactose, a small aliquot of the reaction has ^{14}C-galactose-UDP ligand added; the label incorporated into the peptide is separated from the free label by gel filtration on a Toso Haas G3000SW analytical column using PBS buffer (pH 7.1). The radioactive label incorporation into the peptide is quantitated using an in-line radiation detector.

When the reaction is complete, the reaction mixture is purified using a Toso Haas TSK-Gel-3000 preparative column using PBS buffer (pH 7.1) and collecting fractions based on UV absorption. The fractions containing product are combined, concentrated, buffer exchanged and then freeze-dried. The product of the reaction is analyzed using SDS-PAGE and IEF analysis according to the procedures and reagents supplied by Invitrogen. Samples are dialyzed against water and analyzed by MALDI-TOF MS.

Rnase

38. Remodeling high mannose N-glycans to hybrid and complex N-glycans: Bovine pancreatic RNase

This example sets forth the preparation of bovine pancreas RNase with hybrid or complex N-glycans. The high mannose N-linked glycans of the RNase are enzymatically digested and elaborated to create hybrid N-linked glycans. Additionally, the high mannose N-linked glycans of the RNase are enzymatically digested and elaborated to create complex N-linked glycans.

High mannose structures of *N*-linked oligosaccharides in glycopeptides can be modified to hybrid or complex forms using the combination of α-mannosidases and glycosyltransferases. This example summarizes the results in such efforts using a simple *N*-Glycan as a model substrate.

Ribonuclease B (RNaseB) purified from bovine pancreas (Sigma) is a glycopeptide consisting of 124 amino acid residues. It has a single potential *N*-glycosylation site modified with high mannose structures. Due to its simplicity and low molecular weight (13.7 kDa to 15.5 kDa), ribonuclease B is a good candidate to demonstrate the feasibility of the *N*-Glycan remodeling from high mannose structures to hybrid or complex *N*-linked oligosaccharides. The MALDI-TOF spectrum of RNaseB (Figure 180A) and HPLC profile for the oligosaccharides cleaved from RNaseB by N-Glycanase (Figure 180B) indicated that, other than a small portion of the non-modified peptide, the majority of *N*-glycosylation sites of the peptide are modified with high mannose oligosaccharides consisting of 5 to 9 mannose residues.

Conversion of high mannose N-Glycans to hybrid N-Glycans. High mannose *N*-Glycans were converted to hybrid *N*-Glycans using the combination of α1,2-mannosidase, GlcNAcT-I (β-1,2-*N*-acetyl glucosaminyl transferase), GalT-I (β1,4-galactosyltransfease) and α2,3-sialyltransferase /or α2,6-sialyltransferase as shown in Figure 181.

As an example, high mannose structures in RNaseB were successfully converted to hybrid structures.

$Man_5GlcNAc_2$-R was obtained from $Man_{5-9}GlcNAc_2$-R catalyzed by a single α1,2-mannosidase cloned from *Trichoderma reesei* (Figure 182). RNase B (1 g, about 67 μmol) was incubated at 30°C for 45 hr with 15 mU of the recombinant *T. reesei* α1,2-mannosidase

in MES buffer (50 mM, pH 6.5) in a total volume of 10 mL. $Man_{6-9}GlcNAc_2$-protein structures have been successfully converted to $Man_5GlcNAc_2$-protein with high efficiency by the recombinant mannosidase.

Alternately, $Man_5GlcNAc_2$-R was obtained from $Man_{5-9}GlcNAc_2$-R catalyzed by a single α1,2-mannosidase purified from *Aspergillus saitoi* (Figure 183). RNase B (40 μg, about 2.7 nmol) was incubated at 37°C for 42.5 hr with 25 μU of the commercial *A. saitoi* α1,2-mannosidase (Glyko or CalBioChem) in NaOAC buffer (100 mM, pH 5.0) in a total volume of 20 μl. $Man_{6-9}GlcNAc_2$-protein structures were successfully converted to $Man_5GlcNAc_2$-protein by the commercially available mannosidase. However, a new peak corresponding to the GlcNAc-protein appears in the spectrum, indicating the possible contamination of endoglycosidase H in the preparation. Although several mammalian alpha-mannosidases were required to achieve this step, the fungal α1,2-mannosidase was very efficient to remove all α1,2-linked mannose residues.

GlcNAcT-I then added a GlcNAc residue to the $Man_5GlcNAc_2$-R (Figure 184). The reaction mixture after the *T. reesei* α1,2-mannosidase reaction containing RNase B (600 μg, about 40 nmol) was incubated with non-purified recombinant GlcNAcT-I (34 mU) in MES buffer (50 mM, pH 6.5) containing $MnCl_2$ (20 mM) and UDP-GlcNAc (5 mM) in a total volume of 400 μl. at 37°C for 42 hr. A GlcNAc residue was quantitatively added to $Man_5GlcNAc_2$-protein by the recombinant GlcNAcT-I.

A Gal residue was then added using GalT 1 (Figure 185). The reaction mixture after the GnT-I reaction containing RNase B (120 μg, about 8 nmol) was incubated at 37°C for 20 hr with 3.3 mU of the recombinant GalT-1 in Tris-HCl buffer (100 mM, pH 7.3) containing UDP-Gal (7.5 mM) and $MnCl_2$ (20 mM) in a total volume of 100 μl. A Gal residue was added to about 98% of the GlcNAc-$Man_5GlcNAc_2$-protein by the recombinant GalT 1.

The next step was the addition of a sialic acid using an α2,3-sialyltransferase or an α2,6-sialyltransferase (Figure 186). As an example, ST3Gal III, an α2,3-sialyltransferase was used. The reaction mixture after the GalT-1 reaction containing RNase B (13 μg, about 0.87 nmol) was incubated at 37°C for 16 hr with 8.9 mU of recombinant ST3Gal III in Tris-HCl buffer (100 mM, pH 7.3) containing CMP-Sialic acid (5 mM) and $MnCl_2$ (20 mM) in a total volume of 20 μl. A sialic acid residue was added to about 90% of the Gal-GlcNAc-

$Man_5GlcNAc_2$-protein by recombinant ST3Gal III using CMP-SA as the donor. The yield can be further improved by adjusting the reaction conditions.

For convenience, no purification or dialysis step was required after each reaction described above. More interesting, GalT 1 and ST3Gal III can be combined in a one-pot reaction. Similar yields were obtained as compared with the separate reactions. The reaction mixture after the GlcNAcT-I reaction containing RNase B (60 μg, about 4 nmol) was incubated at 37°C for 20 hr with 1.7 mU of recombinant GalT 1, 9.8 mU of recombinant ST3Gal III in Tris-HCl buffer (100 mM, pH 7.3) containing UDP-Gal (7.5 mM), CMP-sialic acid (5 mM) and $MnCl_2$ (20 mM) in a total volume of 60 μl.

As shown in Figure 187, SA-PEG (10 kDa) was successfully added to the RNaseB. The reaction mixture after the GalT-1 reaction containing RNase B (6.7 μg, about 0.45 nmol) was dialyzed against H_2O for 1 hour at room temperature and incubated at 37°C for 15.5 hours with 55 mU of the recombinant ST3Gal III in Tris-HCl buffer (50 mM, pH 7.3) containing CMP-SA-PEG (10 kDa) (0.25 mM) and $MnCl_2$ (20 mM) in a total volume of 20 μl. PEG-modified sialic acid residues were successfully added to the Gal-GlcNAc-$Man_5GlcNAc_2$-peptide by the recombinant ST3Gal III. The yield can be further improved by adjusting the reaction conditions.

Conversion of high mannose N-Glycans to complex N-Glycans. To achieve this conversion, a GlcNAcβ1,2$Man_3GlcNAc_2$-peptide intermediate is obtained. As shown in Figure 188, there are at least four feasible routes to carry out the reaction from $Man_5GlcNAc_2$-peptide to this intermediate:

Route I: The $Man_5GlcNAc_2$-peptide produced by the fungal α1,2 mannosidase is a substrate of GlcNAc transferase I (GlcNAcT-I, enzyme 2) which adds one GlcNAc. The terminal α1,3- and α1,6-linked mannose residues of $GlcNAcMan_5GlcNAc_2$-peptide is removed by Golgi α-mannosidase II (ManII, enzyme 5). This route is a part of the natural pathway for the processing of *N*-linked oligosaccharides carried out in higher organisms.

Route II: Two mannose residues are first removed by an α-mannosidase (enzyme 6), then a GlcNAc is added by GlcNAcT-I (enzyme 2). Other than its natural acceptor $Man_5GlcNAc_2$-R, GlcNAcT-I can also recognize $Man_3GlcNAc_2$-R as its substrate and add one GlcNAc to the mannose core structure to form $GlcNAcMan_3GlcNAc_2$-peptide.

Route III: The α1,6-linked mannose is removed by an α1,6-mannosidase, followed by the addition of GlcNAc by GlcNAcT-I and removal of the terminal α1,3-linked mannose by an α1,3-mannosidase. From the experimental data obtained, GlcNAcT-I can recognize this $Man_4GlcNAc_2$-peptide as acceptor and add one GlcNAc residue to form $GlcNAcMan_4GlcNAc_2$-peptide.

Route IV: Similar to Route III, α1,3-linked mannose is removed by an α1,3-mannosidase, followed by GlcNAcT-I reaction. Then the terminal α1,6-linked mannose can be removed by an α1,6-mannosidase.

After the function of GlcNAcT-I (responsible for the addition of the GlcNAc β1,2-linked to the α1,3-mannose on the mannose core) and GlcNAcT-II (responsible for the addition of a second GlcNAc β1,2-linked to the α1,6-mannose on the mannose core), the $GlcNAc_2Man_3GlcNAc_2$-peptide can be processed by GalT 1 and sialyltransferase to form bi-antennary complex N- Glycans. Other GlcNAc transferases such as GlcNAcT-IV, GlcNAcT-V, and/or GlcNAcT-VI (Figure 188 and Figure 189) can also glycosylate the $GlcNAc_2Man_3GlcNAc_2$-peptide. Additional glycosylation by the GalT 1 and sialyltransferases will form multi-antennary complex N-glycans. The enzyme GlcNAcT-III catalyzes the insertion of a bisecting GlcNAc, thus preventing the actions of ManII and subsequent action of transferases GlcNAcT-II, GlcNAcT-IV and GlcNAcT-V.

Tissue-Type Plasminogen Activator (TPA)

39. Fucosylation of TPA to create Sialyl Lewis X

This example sets forth the preparation of Tissue Tissue-type Plasminogen Activator (TPA) with N-linked sialyl Lewis X antigen.

Sialylation. TPA expressed in mammalian cells will often contain a majority of the glycans terminating in sialic acid, but to ensure complete sialylation, it would be beneficial to first perform an *in vitro* sialylation. TPA in a suitable buffer (most preferably between pH 5.5 and 9, for example Tris buffered saline, pH 7.2) is incubated with CMP sialic acid and sialyltransferase for a time sufficient to convert any glycans lacking sialic acid to sialylated species. Typical conditions would be 1 mg/mL TPA, 3 mM CMP sialic acid, 0.02 U/mL ST3Gal3, 32°C for 24 hours. Microbial growth can be halted either by sterile filtration or the

inclusion of 0.02% sodium azide. The TPA concentration is most preferably in the range 0.1 mg/mL up to the solubility limit of the peptide. The concentration of CMP-SA should be sufficient for there to be excess over the available sites, and might range from 50 μM up to 50 mM, and the temperature from 2°C up to 40°C. The time required for complete reaction will depend on the temperature, the relative amounts of enzyme to acceptor substrate, the donor substrate concentration, and the pH. Other sialyltransferases that may be capable of adding sialic acid in 2,3 linkage include ST3Gal4; microbial transferases could also be used.

Fucosylation. Typical conditions for fucosylation would be 1 mg/mL TPA, 3 mM GDP-fucose, 0.02 U/mL FTVI, 5 mM $MnCl_2$, 32°C for 24H in Tris buffered saline. Microbial growth can be halted either by sterile filtration or the inclusion of 0.02% sodium azide. The TPA concentration is most preferably in the range 0.1 mg/mL up to the solubility limit of the peptide. The concentration of GDP-fucose should be sufficient for there to be excess over the available sites, and might range from 50 μM up to 50 mM, and the temperature from 2°C up to 40°C. The time required for complete reaction will depend on the temperature, the relative amounts of enzyme to acceptor substrate, the donor substrate concentration, and the pH. Other fucosyltransferases that may be capable of making sialyl Lewis x include FTVII, FTV, FTIII, as well as microbial transferases could also be used.

40. Trimming of high mannose to tri-mannose core structure: Tissue-type Plasminogen Activator produced in CHO

This example sets forth the preparation of Tissue-type Plasminogen Activator with a trimannose core by trimming back from a high mannose glycan.

Tissue-type plasminogen activator (TPA) is currently produced in Chinese Hamster Ovary (CHO) cells and contains a low amount of high mannose N-linked oligosaccharide. The mannoses can be trimmed down using a variety of the specific mannosidases. The first step is to generate Man5GlcNAc2(Fuc0-1) from Man9GlcNAc2(Fuc0-1). This can be done using mannosidase I. Then either GlcNAcT1 (GlcNAc transferase I) is used to make GlcNAc1Man5GlcNAc2(Fuc0-1) or Mannosidase III is used to make Man3GlcNAc2(Fuc0-1). From Man3GlcNAc2(Fuc0-1), GlcNAc1Man3GlcNAc2(Fuc0-1) can be produced using GlcNAcT1 or from GlcNAc1Man5GlcNAc2(Fuc0-1), GlcNAc1Man3GlcNAc2(Fuc0-1) can be produced using Mannosidase II. GlcNAc1Man3GlcNAc2(Fuc0-1) is then converted into

GlcNAc2Man3GlcNAc2(Fuc0-1) using GlcNAcTransferase II (GlcNAcTII). The two terminal GlcNAc residues are then galactosylated using GalTI and then sialylated with SA-PEG using ST3GalIII.

Conversely, TPA can be produce in yeast or fungal systems. Similar processing would be required for fungal derived material.

41. Generation and PEGylation of GlcNAc-ASN structures: TPA produced in Yeast

This example sets forth the preparation of PEGylated GlcNAc-Asn structures on a peptide such as TPA expressed in yeast.

Yeast expression is expected to result in a TPA which contains a single N-linked mannan-type structure. This recombinant glycoprotein is first treated with endoglycosidase H to generate GlcNAc structures on the asparagine (Asn) residues on the peptide.

The GlcNAc-Asn structures on the peptide/protein backbone are then be modified with galactose or galactose-PEG using UDP-galactose or UDP-galactose-6-PEG, respectively, and a galactosyltransferase such as GalT1. In one case, the galactose-PEG is the terminal residue. In the second case, the galactose is further modified with SA-PEG using a CMP-SA-PEG donor and a sialyltransferase such as ST3GalIII. In another embodiment, the GlcNAc-Asn structures on the peptide/protein backbone may be galactosylated and sialylated as described above, and then further sialylated using CMP-SA-PEG and an α2,8-sialyltransferase such as the enzyme encoded by the *Campylobacter jejuni* cst-II gene.

Transferrin

42. GlycoPEGylation of Transferrin

This example sets forth the preparation of asialotransferrin and its sialylation with PEG-CMP-sialic acid.

Preparation of Asialo-transferrin. Human-derived holo-Transferrin, (10 mg) was dissolved in 500 μL of 50 mM NaOAc, 5 mM $CaCl_2$, pH 5.5. To this solution was added 500 mU Neuraminidase II (*Vibrio cholerae*) and the reaction mixture was shaken gently for 20.5 hours at 37 °C. The reaction mixture was added to the prewashed N-(*p*-

aminophenyl)oxamic acid-agarose conjugate (600 μL) and the washed beads gently rotated for 24 hours at 4 °C. The mixture was centrifuged at 10,000 rpm and the supernatant was collected. The reaction mixture was adjusted to 5 mM EDTA by addition of 100 μL of 30 mM EDTA to the washed beads, which were gently rotated for 20 hours at 4 °C. The suspension was centrifuged for 2 minutes at 10,000 rpm and the supernatant was collected. The beads were washed 5 times with 0.35 mL of 50 mM NaOAc, 5 mM $CaCl_2$, 5 mM EDTA, pH 5.5 and all supernatants were pooled. The enzyme solution was dialyzed twice at 4 °C into 15 mM Tris-HCl, 1 M NaCl, pH 7.4. 0.3 mL of the transferrin solution (3.3 mL total) was removed and dialyzed twice against water. The remainder was dialyzed twice more at 4 °C against phosphate buffered saline. The dialyzed solution was stored at –20 ° C. Protein samples were analyzed by IEF Electrophoresis. Samples (9 μL, 25 μg) were diluted with 16 μL Tris buffer and mixed with 25 μL of the sample loading buffer and applied to Isoelectric Focusing Gels (pH 3-7). Gels were run and fixed using standard procedures. Gels were stained with Colloidal Blue Stain.

Sialyl-PEGylation of asialo-Transferrin. Desialylated transferrin (250 μg) and CMP-sialic acid or CMP-SA-PEG (1 kDa or 10 kDa)(0.05 μmol) were dissolved in 69 μL 50 mM Tris-HCl, 0.15 M NaCl, 0.05% NaN_3, pH 7.2 in 1.5 mL plastic tubes. The tubes were vortexed briefly and 100 mU ST3Gal3 (90 μ L) were added (total volume 250 μ L). The tubes were vortexed again and mixed gently for 24 hours at 32 °C. The reactions were stopped by freezing at –80 °C. Novex Tris-Glycine 8-16% 1 mm gels were used for SDS PAGE analysis (Figure 190). Samples (25 μL, 25 μg) were mixed with 25 μL of sample loading buffer and 0.4 μL of β-mercaptoethanol and heated for 6 minutes at 85 °C. Gels were run using standard conditions and stained with Colloidal Blue Stain. IEF gels were also performed as described above Figure 191). Samples were also dialyzed against water analyzed by MALDI-TOF.

Results. MALDI was also performed. Native transferrin (78729); asialotransferrin (78197); resialylated transferrin (79626/80703); with SA-PEG 1k (79037 (1); 80961 (2); 82535 (3); 84778 (4)); with SA-PEG 5k (90003 (2); 96117 (3); 96117 (4)); with SA-PEG 10k (100336 (2); 111421 (3); 122510 (4)).

43. Transferrin-GDNF

This example sets forth the procedures for the glycoconjugation of proteins, and in particular, transferrin is glycoconjugated to GDNF. Transferrin-SA-Linker-Gal-UDP is prepared from transferrin. The galactose residue is removed from GNDF glycans, and Transferrin-SA-Linker-Gal-UDP is conjugated to GNDF glycans using a galactosyltransferase.

Preparation of agalacto-GDNF. GDNF produced in NSO cells (NSO murine myeloma cells) is dissolved at 2.5 mg/mL in 50 mM Tris 50 mM Tris-HCl pH 7.4, 0.15 M NaCl, and is incubated with 300 mU/mL beta-galactosidase-agarose conjugate for 16 hours at 32°C. To monitor the reaction a small aliquot of the reaction is diluted with the appropriate buffer and a IEF gel performed according to Invitrogen procedures. The mixture is centrifuged at 10,000 rpm and the supernatant is collected. The supernatant is dialyzed at 4 °C against 50 mM Tris –HCl pH 7.4, 1 M NaCl, 0.05% NaN_3 and then twice more against 50 mM Tris –HCl pH 7.4, 1 M NaCl, 0.05% NaN_3. The dialyzed solution is then concentrated using a Centricon Plus 20 centrifugal filter and stored at –20 °C. The conditions for the IEF gel are run according to the procedures and reagents provided by Invitrogen. Samples are dialyzed against water and analyzed by MALDI-TOF MS.

Preparation of Transferrin-SA-Linker-Gal-UDP. Asialo-transferrin is dissolved at 2.5 mg/mL in 50 mM Tris-HCl, 0.15 M NaCl, 0.05% NaN_3, pH 7.2. The solution is incubated with CMP-sialic acid-linker-Gal-UDP (molar amount to add 1 molar equivalent of nucleotide sugar to transferrin) and 0.1 U/mL of ST3Gal3 at 32°C for 2 days. To monitor the incorporation of sialic acid, a small aliquot of the reaction has ^{14}C-SA-UDP ligand added; the label incorporated into the peptide is separated from the free label by gel filtration on a Toso Haas G3000SW analytical column using PBS buffer (pH 7.1). The radioactive label incorporation into the peptide is quantitated using an in-line radiation detector.

The solution is incubated with 5 mM CMP-sialic acid and 0.1 U/mL of ST3Gal3 (to cap any unreacted transferrin glycans) at 32°C for 2 days. The incorporation into the peptide is quantitated using an in-line UV detector. After 2 days, the reaction mixture is purified using a Toso Haas G3000SW preparative column using PBS buffer (pH 7.1) and collecting fractions based on UV absorption. The product of the reaction is analyzed using SDS-PAGE

and IEF analysis according to the procedures and reagents supplied by Invitrogen. Samples are dialyzed against water and analyzed by MALDI-TOF MS.

Preparation of Transferrin-SA-Linker-Gal-GDNF. The transferrin-SA-Linker-Gal-UDP prepared as described above is dissolved at 2.5 mg/mL in 50 mM Tris-HCl, 0.15 M NaCl, 5 mM $MnCl_2$, 0.05% NaN_3, pH 7.2. The solution is incubated with 2.5 mg/mL agalacto-GDNF and 0.1 U/mL of galactosyltransferase at 32°C for 2 days. To monitor the incorporation of galactose, a small aliquot of the reaction has ^{14}C-galactose-UDP ligand added; the label incorporated into the peptide is separated from the free label by gel filtration on a Toso Haas G3000SW analytical column using PBS buffer (pH 7.1). The radioactive label incorporation into the peptide is quantitated using an in-line radiation detector.

When the reaction is complete, the solution is incubated with 5 mM UDP-Gal and 0.1 U/mL of galactosyltransferase (to cap any unreacted transferrin glycans) at 32°C for 2 days followed by addition of 5 mM CMP-SA and 0.1 U/mL of ST3Gal3. After 2 additional days, the reaction mixture is purified using a Toso Haas G3000SW preparative column using PBS buffer (pH 7.1) collecting fractions based on UV absorption. The product of the reaction is analyzed using SDS-PAGE and IEF analysis according to the procedures and reagents supplied by Invitrogen. Samples are dialyzed against water and analyzed by MALDI-TOF MS.

The disclosures of each and every patent, patent application, and publication cited herein are hereby incorporated herein by reference in their entirety.

While this invention has been disclosed with reference to specific embodiments, it is apparent that other embodiments and variations of this invention may be devised by others skilled in the art without departing from the true spirit and scope of the invention. The appended claims are intended to be construed to include all such embodiments and equivalent variations.

What is claimed:

1. An EPO peptide comprising one or more glycans, having a glycoconjugate molecule covalently attached to said peptide.

2. The EPO peptide of claim 1, wherein said one or more glycans is a monoantennary glycan.

3. The EPO peptide of claim 1, wherein said one or more glycans is a biantennary glycan.

4. The EPO peptide of claim 1, wherein said one or more glycans is a triantennary glycan.

5. The EPO peptide of claim 1, wherein said one or more glycans is at least a triantennary glycan.

6. The EPO peptide of claim 1, wherein said one or more glycans comprises at least two glycans comprising a mixture of mono or multiantennary glycans.

7. The EPO peptide of claim 1, wherein said one or more glycans is selected from an N-linked glycan and an O-linked glycan.

8. The EPO peptide of claim 1, wherein said one or more glycans is at least two glycans selected from an N-linked and an O-linked glycan.

9. The EPO peptide of claim 1, wherein said peptide is expressed in a cell selected from the group consisting of a prokaryotic cell and a eukaryotic cell.

10. The EPO peptide of claim 9, wherein said eukaryotic cell is selected from the group consisting of a mammalian cell, an insect cell and a fungal cell.

11. The EPO peptide of claim 10, wherein said fungal cell is a yeast cell.

12. A glycoPEGylated EPO peptide comprising an EPO peptide and at least one glycan and at least one poly(ethylene glycol) molecule covalently attached to said glycan,

wherein said poly(ethylene glycol) molecule is added to said EPO peptide using a glycosyltransferase.

13. The glycoPEGylated EPO peptide of claim 12, comprising at least one mono-antennary glycan.

14. The glycoPEGylated EPO peptide of claim 12, wherein all of said glycans are N-linked and are mono-antennary.

15. The glycoPEGylated EPO peptide of claim 12, wherein all of said glycans are N-linked and at least one of said glycans comprise said poly(ethylene glycol).

16. The glycoPEGylated EPO peptide of claim 15, wherein more than one of said glycans comprises said poly(ethylene glycol).

17. The glycoPEGylated EPO peptide of claim 12, wherein all of said glycans are N-linked and all of said glycans comprise said poly(ethylene glycol).

18. The glycoPEGylated EPO peptide of claim 12, comprising at least three mono-antennary glycans having said poly(ethylene glycol) covalently attached thereto.

19. A glycoPEGylated EPO peptide, wherein said EPO peptide comprises three or more glycans.

20. The glycoPEGylated EPO peptide of claim 9, wherein at least one of said glycans comprises said poly(ethylene glycol) covalently attached thereto.

21. The glycoPEGylated EPO peptide of claim 18, wherein more than one of said glycans comprises said poly(ethylene glycol) covalently attached thereto.

22. The glycoPEGylated EPO peptide of claim 18, wherein all of said glycans comprise said poly(ethylene glycol) covalently attached thereto.

23. The glycoPEGylated EPO peptide of claim 12 wherein said poly(ethylene glycol) is linked to at least one sugar moiety selected from the group consisting of fucose (Fuc), N-acetylglucosamine (GlcNAc), galactose (Gal) and a sialic acid (SA).

24. The glycoPEGylated EPO peptide of claim 23, wherein said sialic acid is N-acetylneuraminic acid.

25. The glycoPEGylated EPO peptide of claim 12, wherein said EPO peptide does not comprise an O-linked glycan.

26. The glycoPEGylated EPO peptide of claim 12 wherein said EPO peptide comprises at least one O-linked glycan.

27. The glycoPEGylated EPO peptide of claim 26, wherein said O-linked peptide comprises said poly(ethylene glycol) covalently attached thereto.

28. The glycoPEGylated EPO peptide of claim 27, wherein said EPO peptide is recombinantly expressed in a cell.

29. The glycoPEGylated EPO peptide of claim 28, wherein said cell is selected from the group consisting of an insect cell, a fungal cell and a mammalian cell.

30. The glycoPEGylated EPO peptide of claim 29, wherein said fungal cell is a yeast cell.

31. The glycoPEGylated EPO peptide of claim 29, wherein said cell is an insect cell.

32. The glycoPEGylated EPO peptide of claim 29, wherein said cell is a yeast cell.

33. The glycoPEGylated EPO peptide of claim 29, wherein said cell is a mammalian cell.

34. The glycoPEGylated EPO peptide of claim 33, wherein said mammalian cell is a CHO cell.

35. The glycoPEGylated EPO peptide of claim 12, wherein said poly(ethylene glycol) has a molecular weight selected from the group consisting of about 1 kDa, 2 kDa, 5 kDa, 10 kDa, 20 kDa, 30 kDa and 40 kDa.

36. The glycoPEGylated EPO peptide of claim 35, wherein said poly(ethylene glycol) has a molecular weight of 20 kDa.

37. The glycoPEGylated EPO peptide of claim 12, wherein said EPO peptide is selected from the group consisting of a naturally occurring EPO peptide and a mutated EPO peptide.

38. The glycoPEGylated EPO peptide of claim 37, wherein said mutated EPO peptide comprises the amino acid sequence of SEQ ID NO:73 having at least one mutation selected from the group consisting of Arg^{139} to Ala^{139}, Arg^{143} to Ala^{143} and Lys^{154} to Ala^{154}.

39. A method of making a glycoPEGylated EPO peptide, said method comprising the step of:

(a) contacting an EPO peptide with a mixture comprising a nucleotide sugar covalently linked to poly(ethylene glycol) and a glycosyltransferase under conditions sufficient to transfer said poly(ethylene glycol) to said EPO peptide.

40. The method of claim 39, wherein the sugar of said nucleotide sugar is selected from the group consisting of fucose (Fuc), N-acetylglucosamine (GlcNAc), galactose (Gal) and a sialic acid (SA).

41. The method of claim 40, wherein said sialic acid is N-acetylneuraminic acid (NAN).

42. The method of claim 39, wherein said poly(ethylene glycol) has a molecular weight selected from the group consisting of about 1 kDa, 2 kDa, 5 kDa, 10 kDa, 20 kDa, 30 kDa and 40 kDa.

43. The method of claim 42, wherein said poly(ethylene glycol) has a molecular weight of 20 kDa.

44. The method of claim 39, wherein said EPO peptide is recombinantly expressed in a cell.

45. The method of claim 44, wherein said cell is selected from the group consisting of an insect cell, a fungal cell and a mammalian cell.

46. The method of claim 45, wherein said cell is an insect cell.

47. The method of claim 45, wherein said cell is a yeast cell.

48. The method of claim 45, wherein said cell is a mammalian cell.

49. The method of claim 48, wherein said mammalian cell is a CHO cell.

50. The method of claim 39, wherein said EPO peptide is selected from the group consisting of a naturally occurring EPO peptide and a mutated EPO peptide.

51. The method of claim 50, wherein said mature EPO peptide has the sequence of SEQ ID NO:73.

52. The method of claim 50, wherein said mutated EPO peptide comprises the amino acid sequence of SEQ ID NO: 73 having at least one mutation selected from the group consisting of Arg^{139} to Ala^{139}, Arg^{143} to Ala^{143} and Lys^{154} to Ala^{154}.

53. The method of claim 39, wherein before step (a):

(b) contacting said EPO peptide with a mixture comprising a nucleotide-N-acetylglucosamine (GlcNAc) molecule and an N-acetylglucosamine transferase (GnT) for which the nucleotide-GlcNAc is a substrate under conditions sufficient to form a bond between said GlcNAc and said EPO, wherein said GnT is selected from the group consisting of GnT I, GnT II, GnT III, GnT IV, GnT V and GnT VI.

54. The method of claim 53, wherein said mixture comprises one GnT selected from the group consisting of GnT I, GnT II, GnT IV, GnT V and GnT VI.

55. The method of claim 54, wherein said GnT is GnT I.

56. The method of claim 54, wherein said GnT is GnT II.

57. The method of claim 39, wherein said glycoPEGylated EPO peptide comprises at least one mono-antennary glycan.

58. The method of claim 39, wherein the sugar of said nucleotide sugar is galactose and said glycosyltransferase is galactosyl transferase I (GalT I).

59. The method of claim 53, wherein before step (a) but after step (b):

(c) contacting said EPO peptide with a mixture comprising a nucleotide galactose (Gal) and galactosyl transferase I (GalT I) under conditions sufficient to transfer galactose to said EPO peptide.

60. The method of claim 39, wherein in step (a), the sugar of said nucleotide sugar is sialic acid and said glycosyltransferase is a sialyltransferase.

61. The method of claim 60, wherein said sialic acid is N-acetylneuraminic acid (NAN).

62. The method of claim 60, wherein said sialyltransferase is selected from the group consisting of α(2,3)sialyltransferase, α(2,6)sialyltransferase and (2,8)sialyltransferase.

63. A glycoPEGylated EPO peptide made by the method of claim 39.

64. A glycoPEGylated EPO peptide, said EPO peptide comprising the sequence of SEQ ID NO:73.

65. A glycoPEGylated EPO peptide, said EPO peptide comprising the sequence of SEQ ID NO:73 and further comprising a mutation in said sequence.

66. A method of making a glycoPEGylated EPO peptide, said method comprising the steps of:

(a) contacting an EPO peptide with a mixture comprising a nucleotide sugar covalently linked to poly(ethylene glycol) and a glycosyltransferase under conditions sufficient to transfer said poly(ethylene glycol) to said EPO peptide, wherein said glycosyltransferase is a fucosyltransferase.

67. The method of claim 66, wherein said fucosyltransferase is selected from the group consisting of fucosyltransferase I, fucosyltransferase III, fucosyltransferase IV, fucosyltransferase V, fucosyltransferase VI and fucosyltransferase VII.

68. A glycoPEGylated EPO peptide made by the method of claim 66.

69. The method of claim 66, wherein said EPO peptide is expressed in a CHO cell.

70. A method of treating a mammal having anemia, said method comprising administering to said mammal an EPO peptide having one or more glycans having a glycoconjugate molecule attached to said peptide, wherein said EPO peptide is administered in an amount effective to increase the hematocrit level in said mammal.

71. The method of claim 70, wherein said mammal is a human.

72. A method of providing erythropoietin therapy to a mammal, said method comprising administering an effective amount of a glycoPEGylated EPO peptide comprising an EPO peptide and at least one glycan and at least one poly(ethylene glycol) molecule covalently attached to said glycan, wherein said poly(ethylene glycol) molecule is added to said EPO peptide using a glycosyltransferase, wherein said EPO peptide is administered in an amount effective to increase the hematocrit level in said mammal.

73. The method of claim 72, wherein said mammal is a human.

74. A method of treating a mammal having anemia, said method comprising administering to said mammal a glycoPEGylated EPO peptide comprising an EPO peptide and at least one glycan and at least one poly(ethylene glycol) molecule covalently attached to said glycan, wherein said poly(ethylene glycol) molecule is added to said EPO peptide using a glycosyltransferase, wherein said EPO peptide is administered in an amount effective to increase the hematocrit level in said mammal..

75. The method of claim 74, wherein said mammal is a human.

76. The method of claim 75, wherein said anemia is associated with chemotherapy.

77. A method of treating a kidney dialysis patient, said method comprising administering to said patient a glycoPEGylated EPO peptide comprising an EPO peptide and at least one glycan and at least one poly(ethylene glycol) molecule covalently attached to said glycan, wherein said poly(ethylene glycol) molecule is added to said EPO peptide using a glycosyltransferase, wherein said EPO peptide is administered in an amount effective to increase the hematocrit level in said patient.

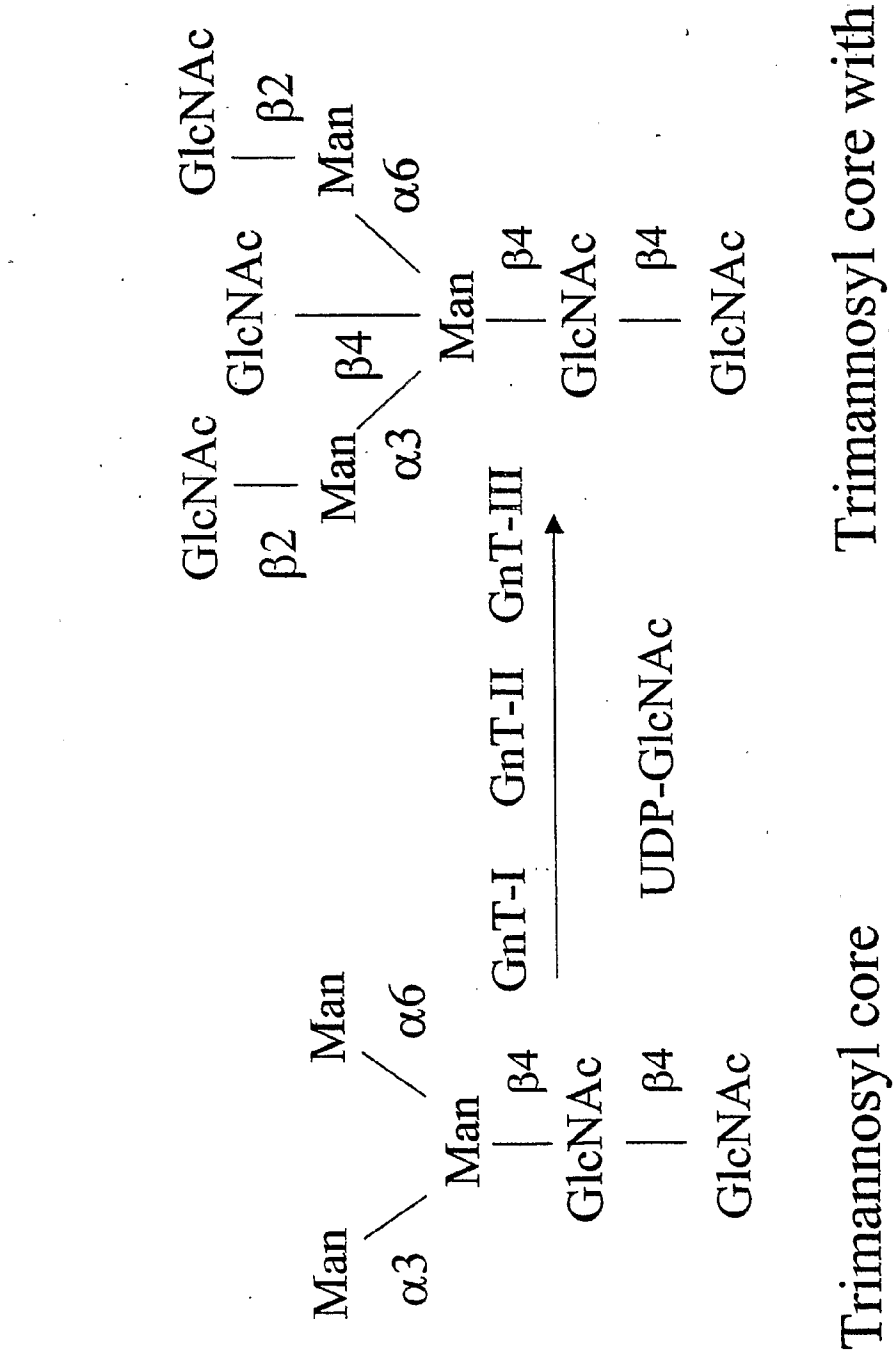

Trimannosyl core
Man
α3
Man
α6
β4
GlcNAc
β4
GlcNAc
GnT-I
GnT-II
GnT-III
UDP-GlcNAc
Trimannosyl core with
Bisecting GlcNAc
GlcNAc
β2
GlcNAc
β2
Man
α3
Man
α6
β4
GlcNAc
β4
GlcNAc

FIG. 1

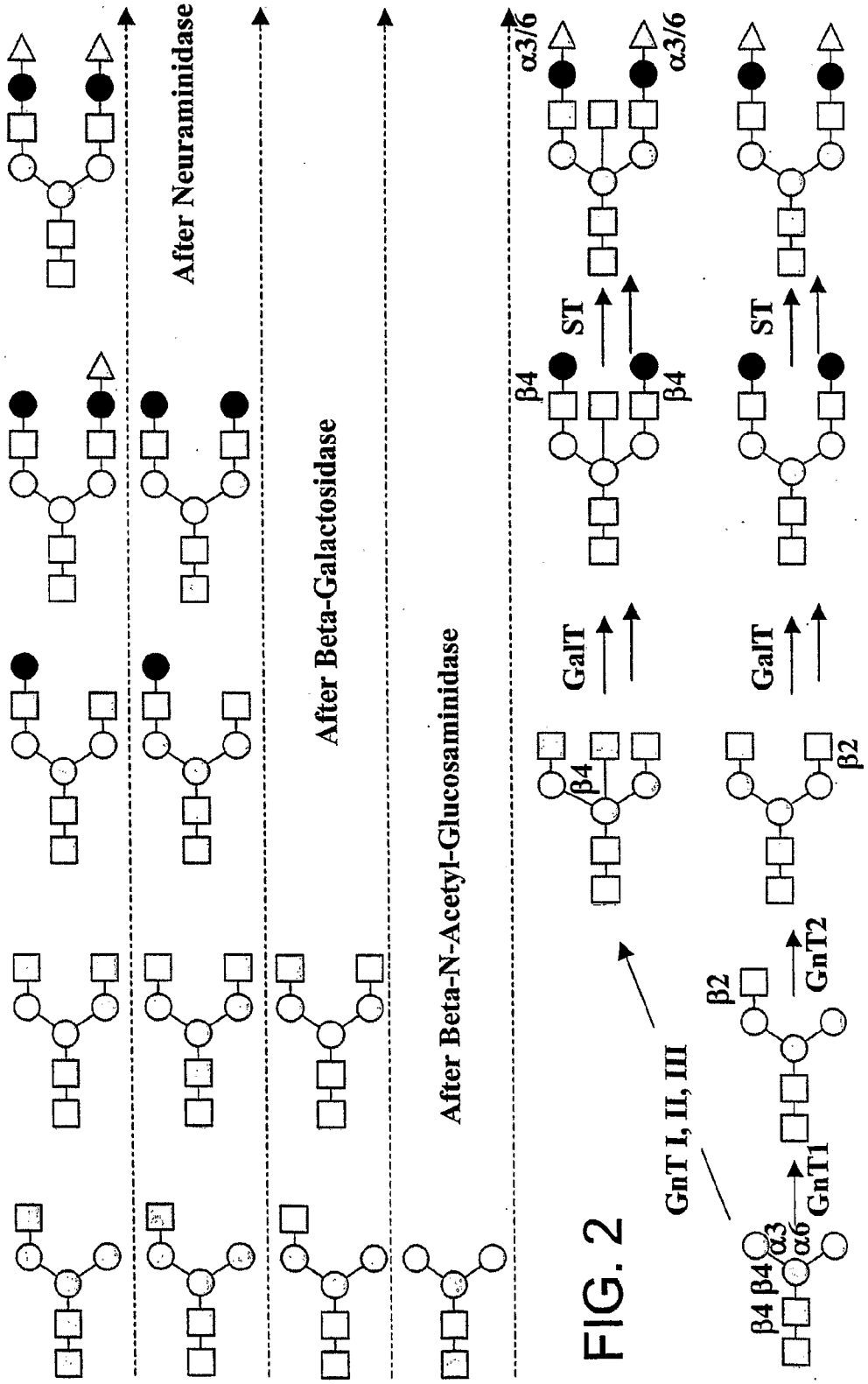

After Neuraminidase
After Beta-Galactosidase
After Beta-N-Acetyl-Glucosaminidase
α3/6
α3/6
β4
ST
β4
ST
GalT
GalT
β4
β2
GnT I, II, III
β2
GnT2
β4 β4
α3
α6
GnT1

FIG. 2

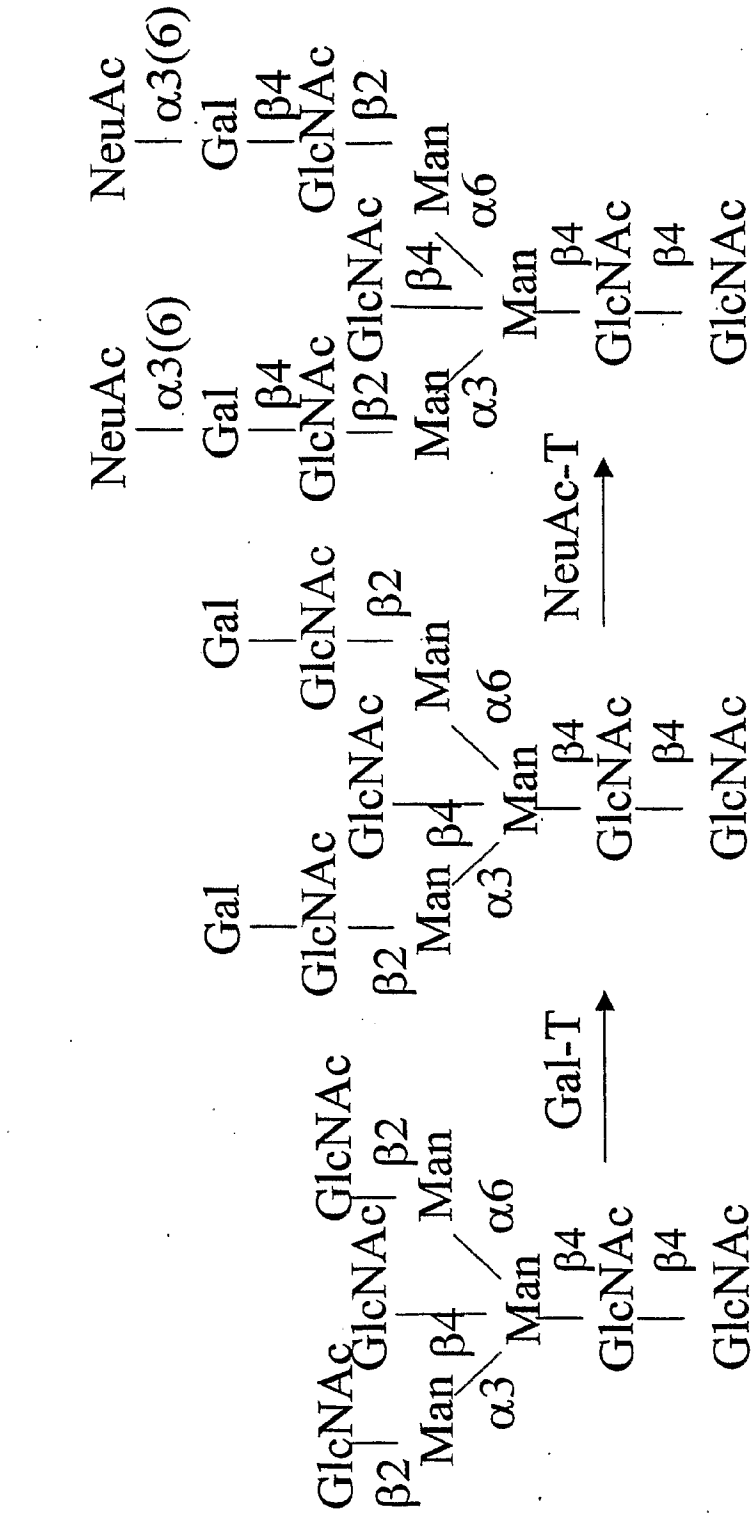

GlcNAc
β2
Man
α3
Man β4
GlcNAc
β2
Man
α6
Man
β4
GlcNAc
β4
GlcNAc
Gal-T
Gal
GlcNAc
β2
Man
α3
Man β4
GlcNAc
β2
Man
α6
Man
β4
GlcNAc
β4
GlcNAc
NeuAc-T
NeuAc
α3(6)
Gal
β4
GlcNAc
β2
Man
α3
Man β4
GlcNAc
β2
Man
α6
Man
β4
GlcNAc
β4
GlcNAc

FIG. 3

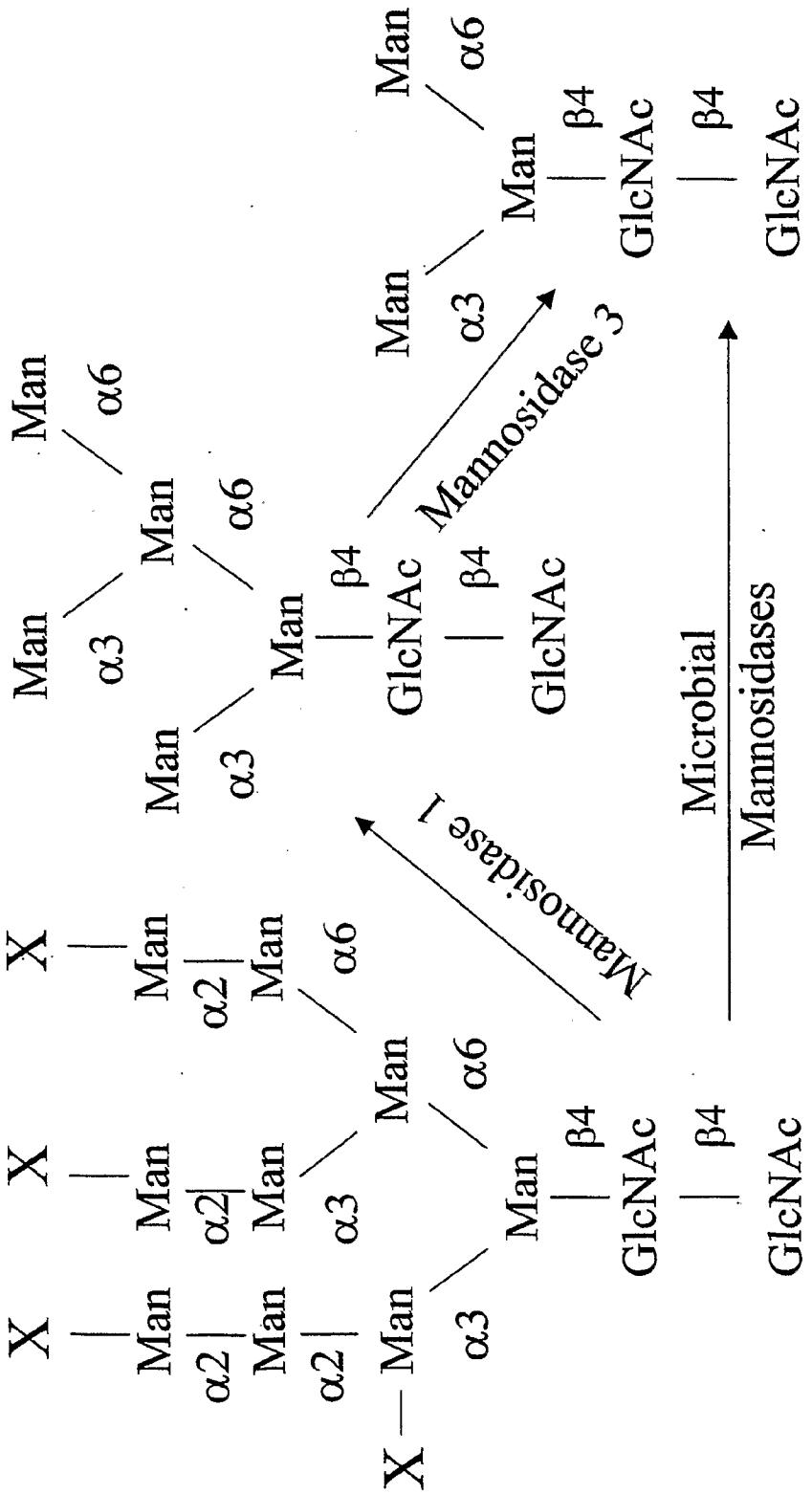

Mannosidase 1
Mannosidase 3
Microbial
Mannosidases
Man
α6
α3
α2
β4
GlcNAc
X

FIG. 4

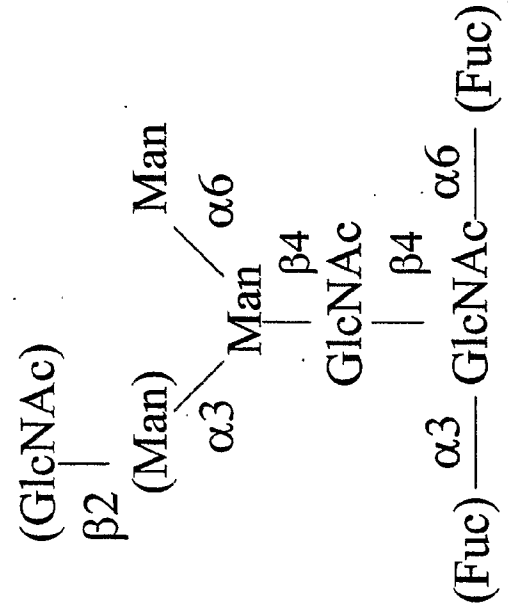

(GlcNAc)
β2
Man
α3
Man
α6
Xyl
β2
Man
β4
GlcNAc
β4
Fuc
α3
GlcNAc

FIG. 5

(GlcNAc)
β2
(Man)
α3
Man
α6
Man
β4
GlcNAc
β4
(Fuc)
α3
GlcNAc
α6
(Fuc)

FIG. 6

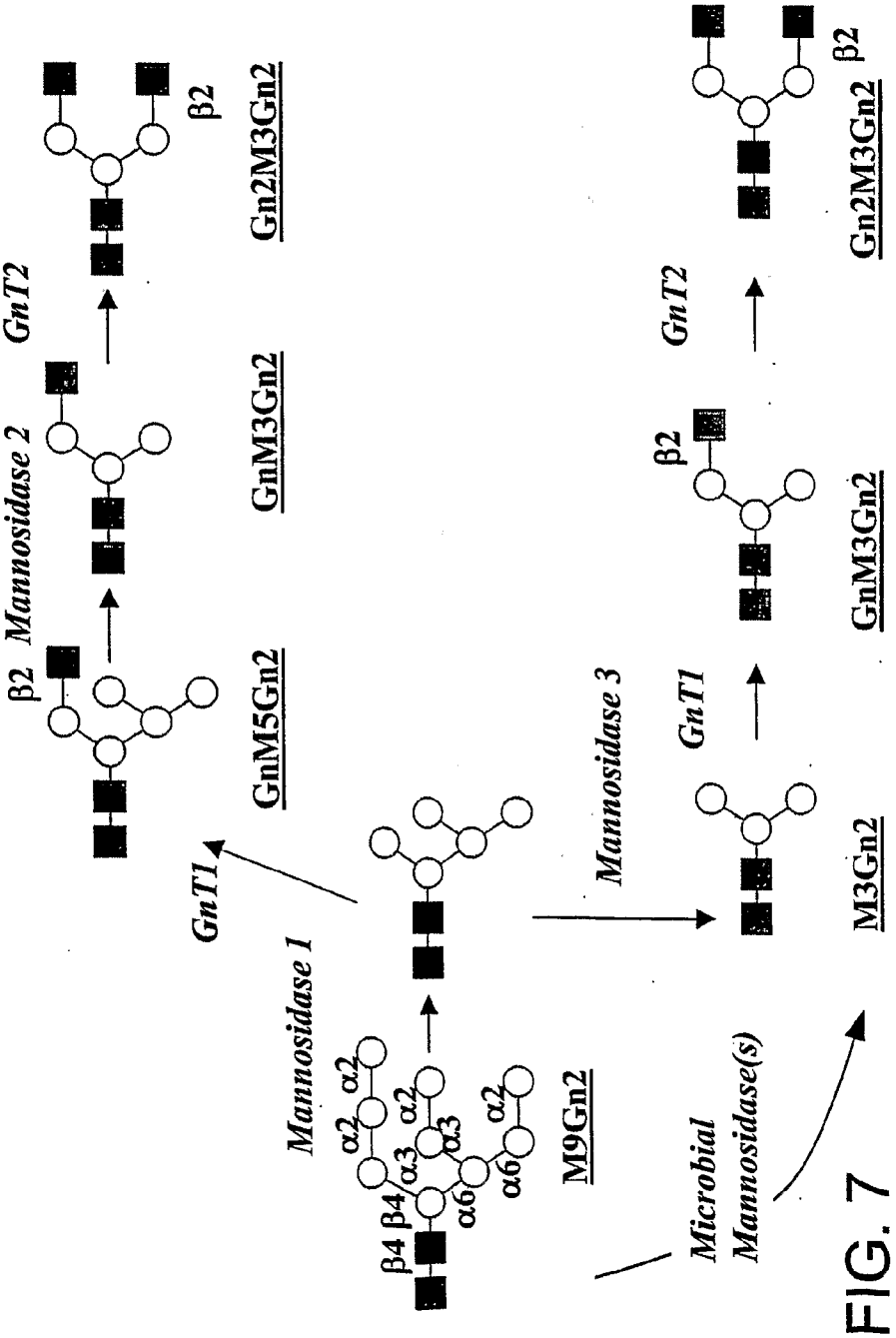

Microbial Mannosidase(s)
M9Gn2
Mannosidase 1
GnT1
GnM5Gn2
β2 Mannosidase 2
GnT2
β2
Gn2M3Gn2
GnM3Gn2
Mannosidase 3
GnT1
M3Gn2
β2
GnT2
β2
Gn2M3Gn2
GnM3Gn2

FIG. 7

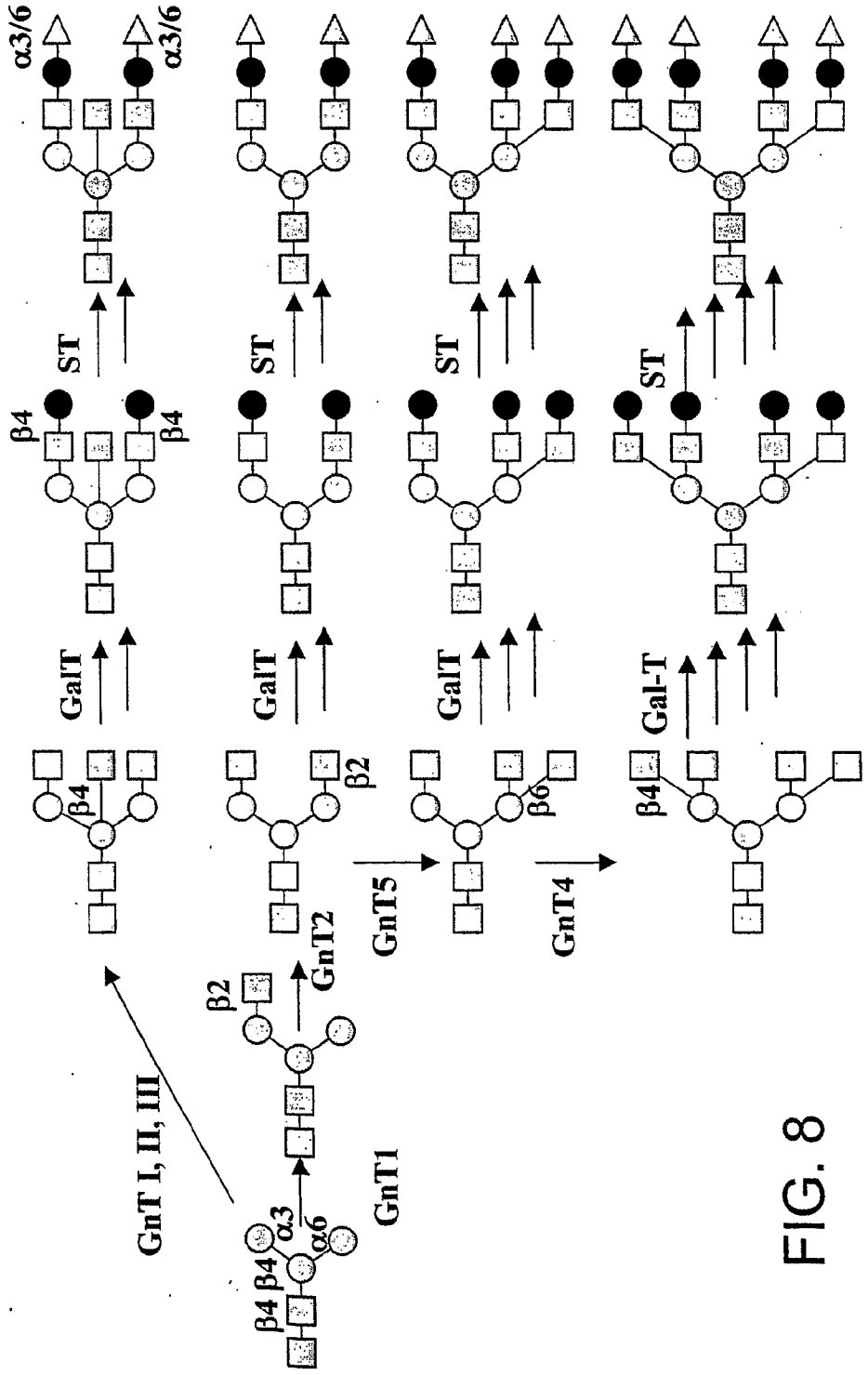

α3/6
α3/6
ST
β4
β4
GalT
β4
β2
GnT2
GnT5
β6
GnT4
Gal-T
β4
β2
GnT I, II, III
GnT1
β4 β4
α3
α6

FIG. 8

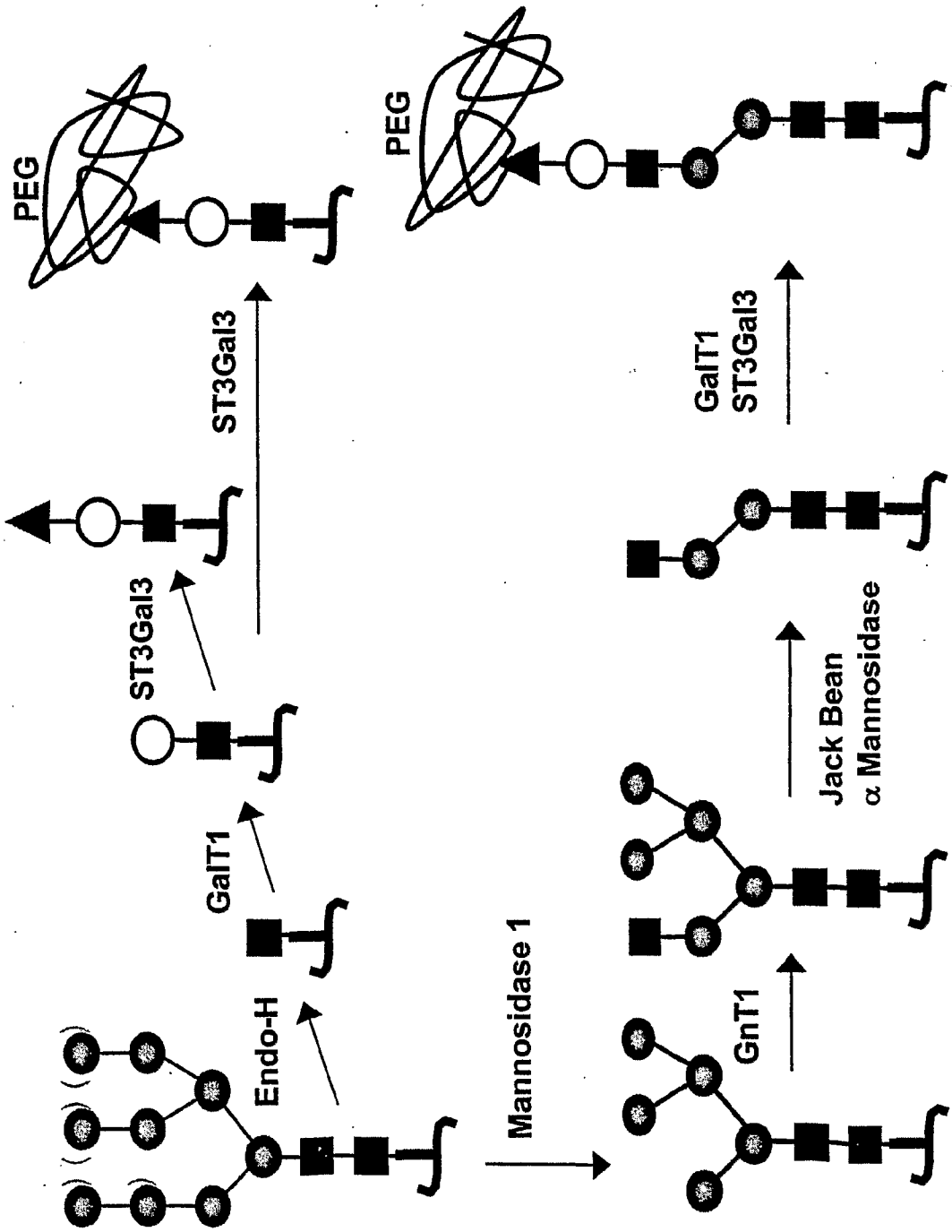

Endo-H
GalT1
ST3Gal3
ST3Gal3
PEG
Mannosidase 1
GnT1
Jack Bean
α Mannosidase
GalT1
ST3Gal3
PEG

FIG. 9

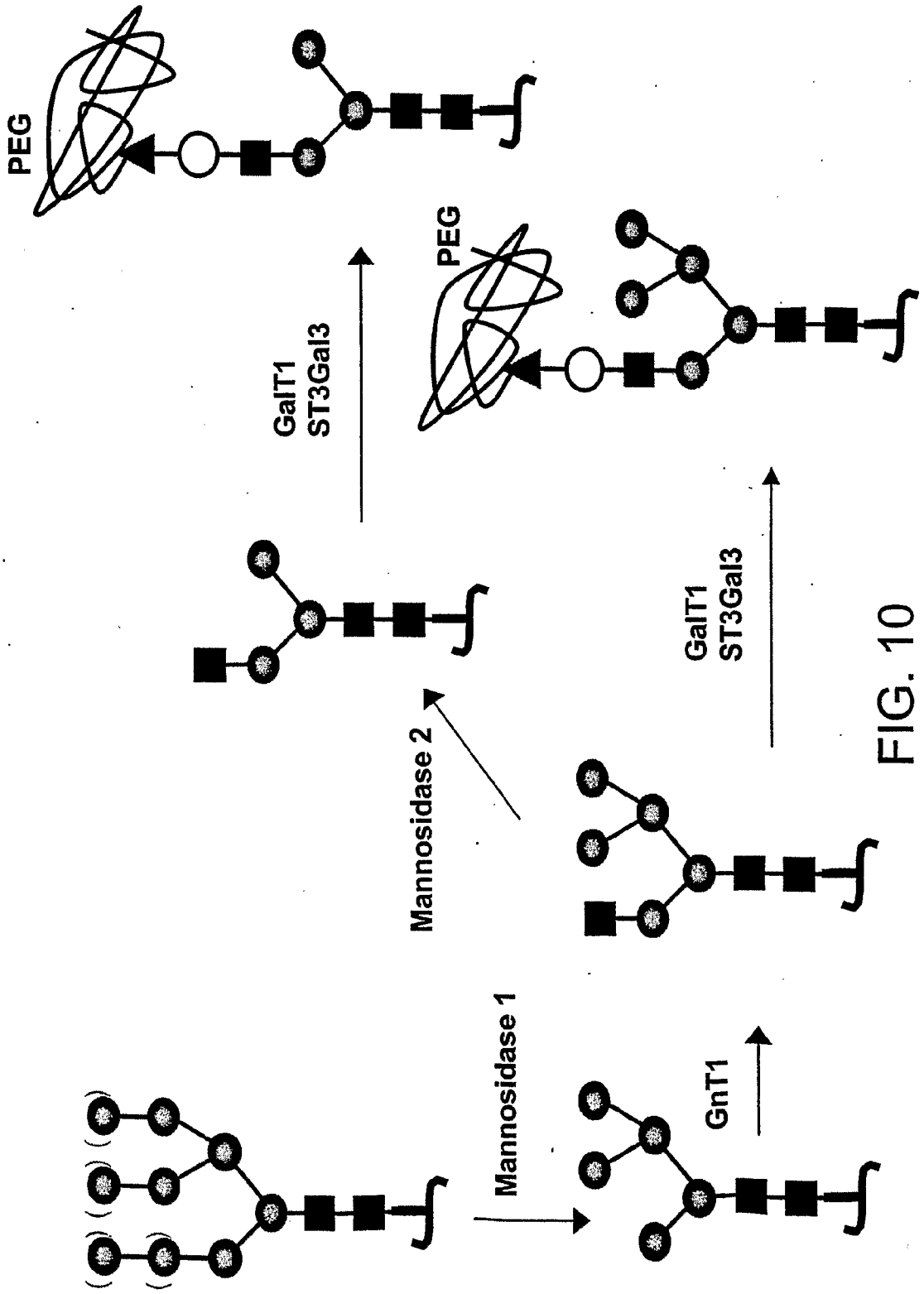

PEG
GalT1
ST3Gal3
PEG
GalT1
ST3Gal3
Mannosidase 2
Mannosidase 1
GnT1

FIG. 10

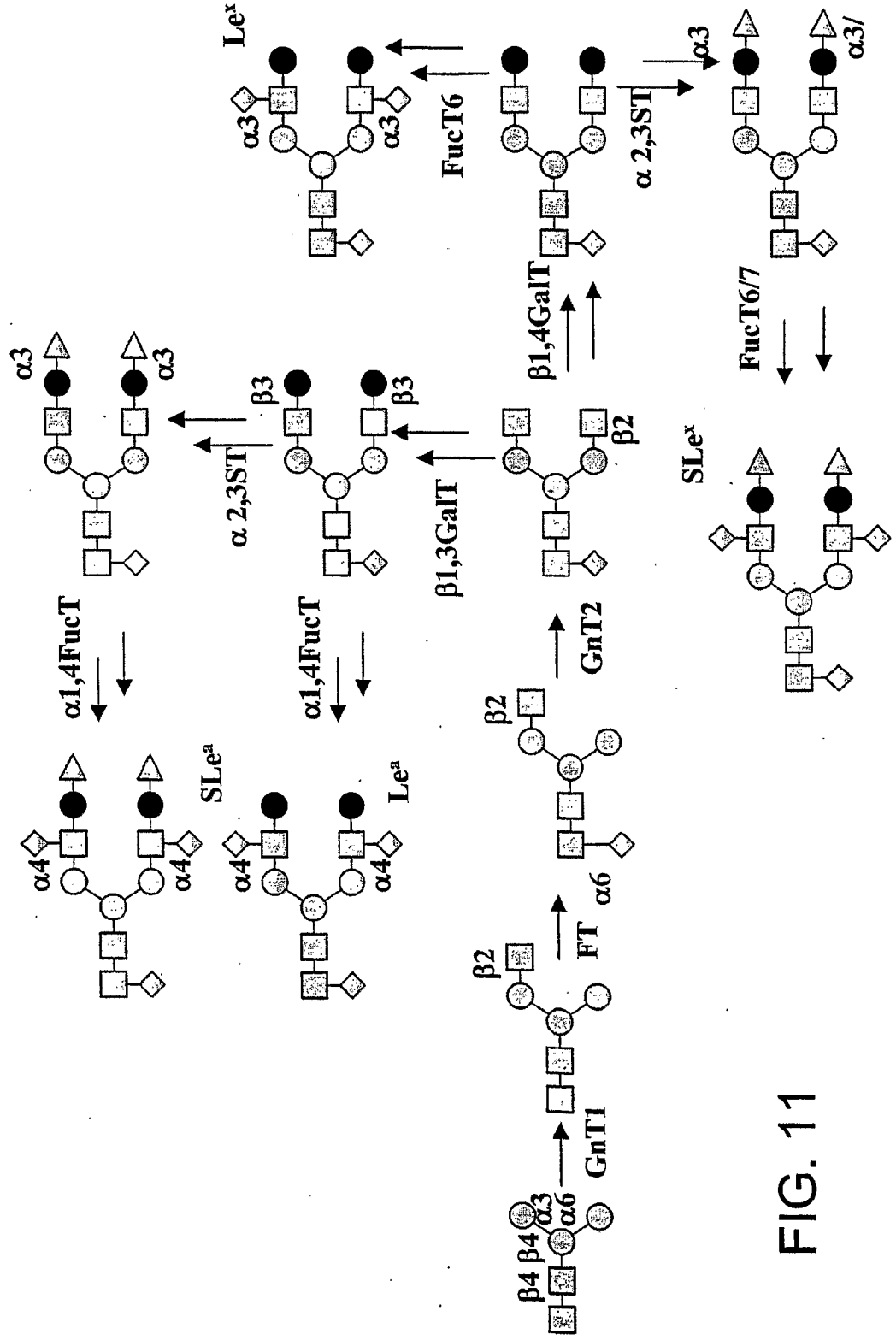

Le^x
α3
α3
FucT6
β1,4GalT
α 2,3ST
α3
α3/
FucT6/7
SLe^x
α3
α3
β3
β3
α 2,3ST
β1,3GalT
β2
α1,4FucT
α1,4FucT
GnT2
β2
SLe^a
Le^a
α4
α4
α4
α4
β2
FT
α6
β4 β4
α3
α6
GnT1

FIG. 11

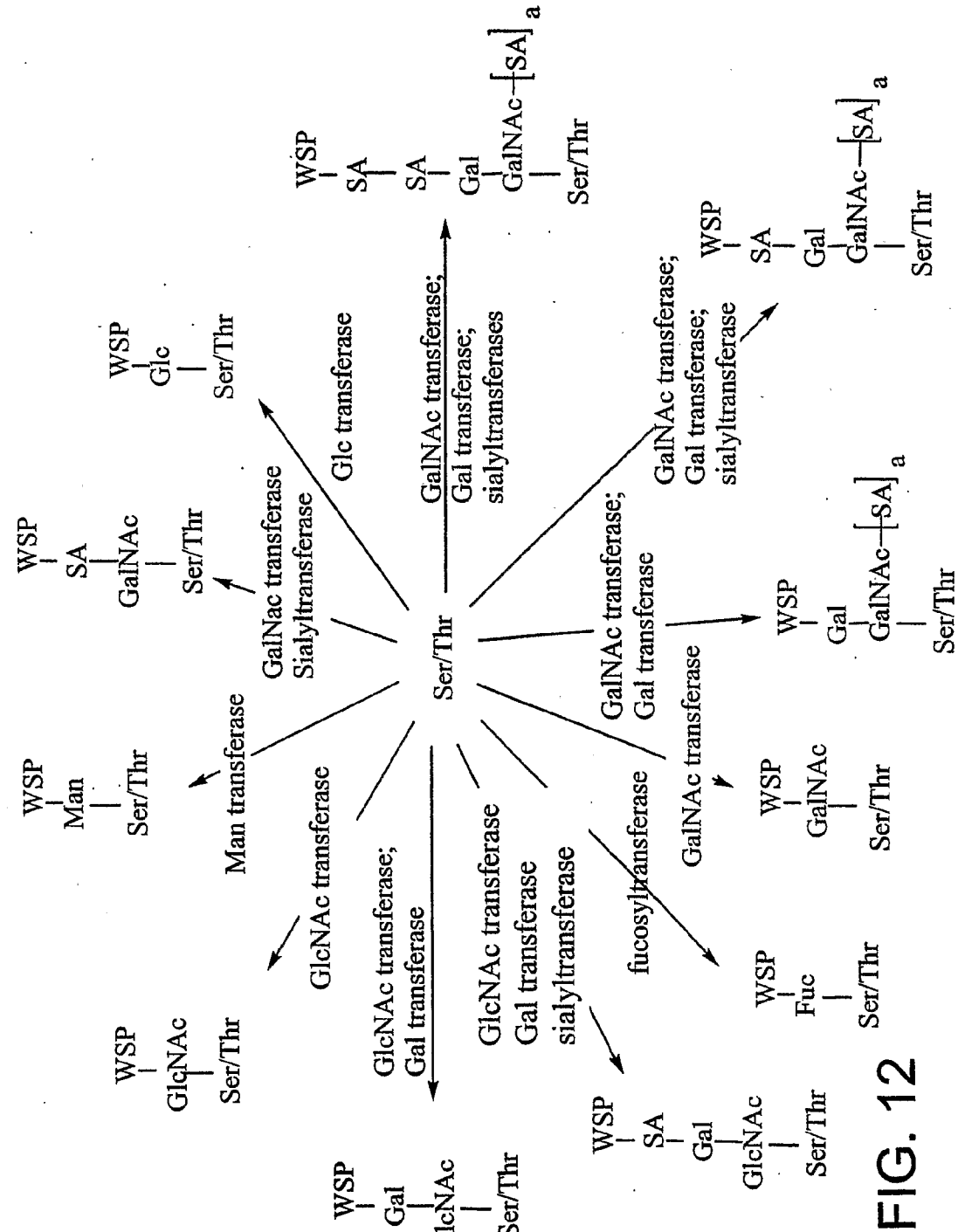
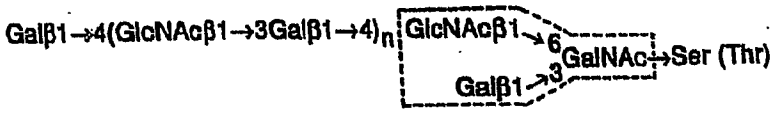

WSP
Man
Ser/Thr
Man transferase
WSP
SA
GalNAc
Ser/Thr
GalNac transferase
Sialyltransferase
WSP
Glc
Ser/Thr
Glc transferase
WSP
SA
SA
Gal
GalNAc—[SA]a
Ser/Thr
GalNAc transferase;
Gal transferase;
sialyltransferases
WSP
GlcNAc
Ser/Thr
GlcNAc transferase
WSP
Gal
GlcNAc
Ser/Thr
GlcNAc transferase;
Gal transferase
Ser/Thr
GlcNAc transferase
Gal transferase
sialyltransferase
GalNAc transferase;
Gal transferase
WSP
SA
Gal
GlcNAc
Ser/Thr
fucosyltransferase
WSP
Fuc
Ser/Thr
GalNAc transferase
WSP
GalNAc
Ser/Thr
GalNAc transferase;
Gal transferase;
sialyltransferase
WSP
SA
Gal
GalNAc—[SA]a
Ser/Thr
WSP
Gal
GalNAc—[SA]a
Ser/Thr

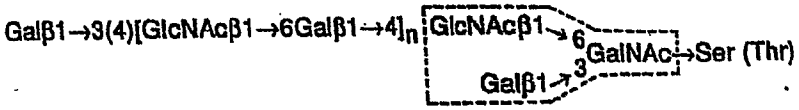
FIG. 12

Core 1

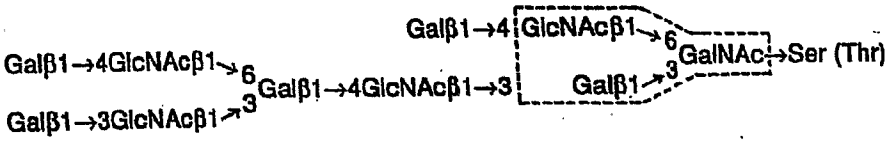
Core 2



n = 0 ~ 2



n = 0 ~ 3



Core 3

Core 4

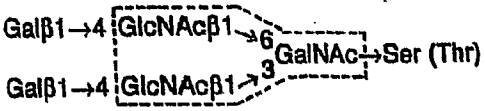
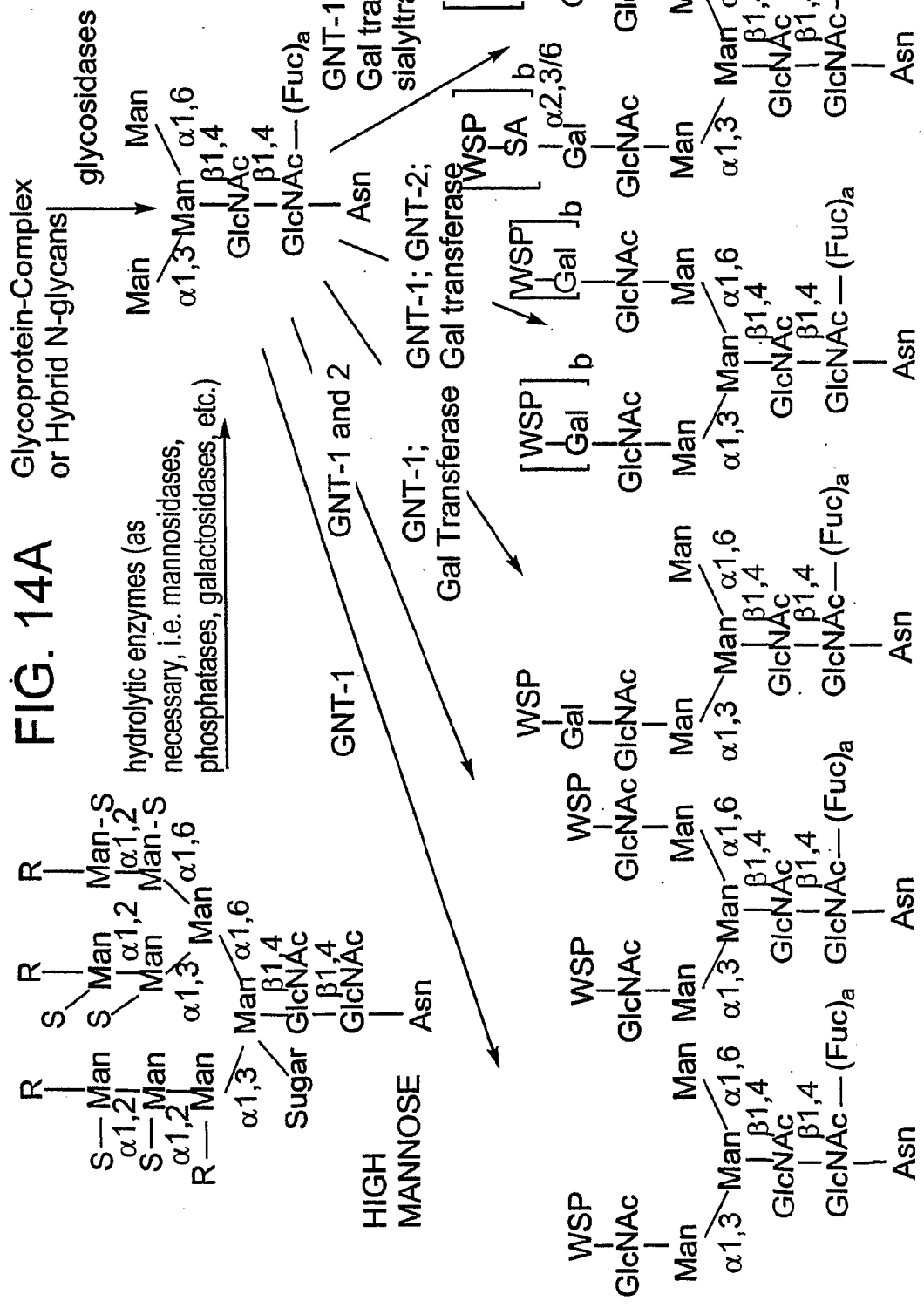


FIG. 13

FIG. 14A


Glycoprotein-Complex
or Hybrid N-glycans
glycosidases
hydrolytic enzymes (as
necessary, i.e. mannosidases,
phosphatases, galactosidases, etc.)
HIGH
MANNOSE
GNT-1
GNT-1 and 2
GNT-1; GNT-2;
Gal Transferase
GNT-1; GNT-2;
Gal transferase;
sialyltranferase
Sugar
Asn
WSP
SA
Gal
GlcNAc
Man
(Fuc)a

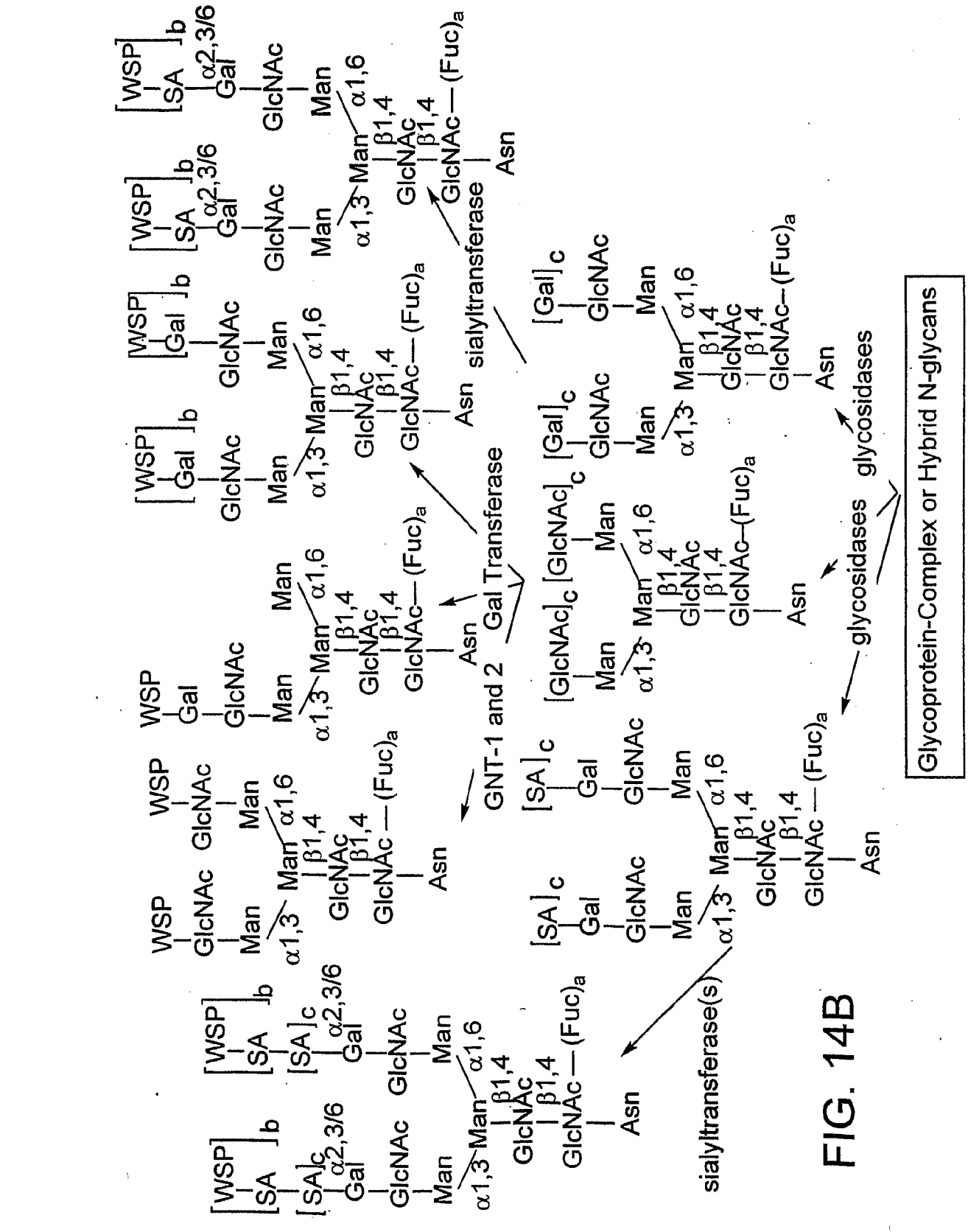

GNT-1 and 2
Gal Transferase
sialyltransferase
sialyltransferase(s)
glycosidases
glycosidases
Glycoprotein-Complex or Hybrid N-glycans

FIG. 14B

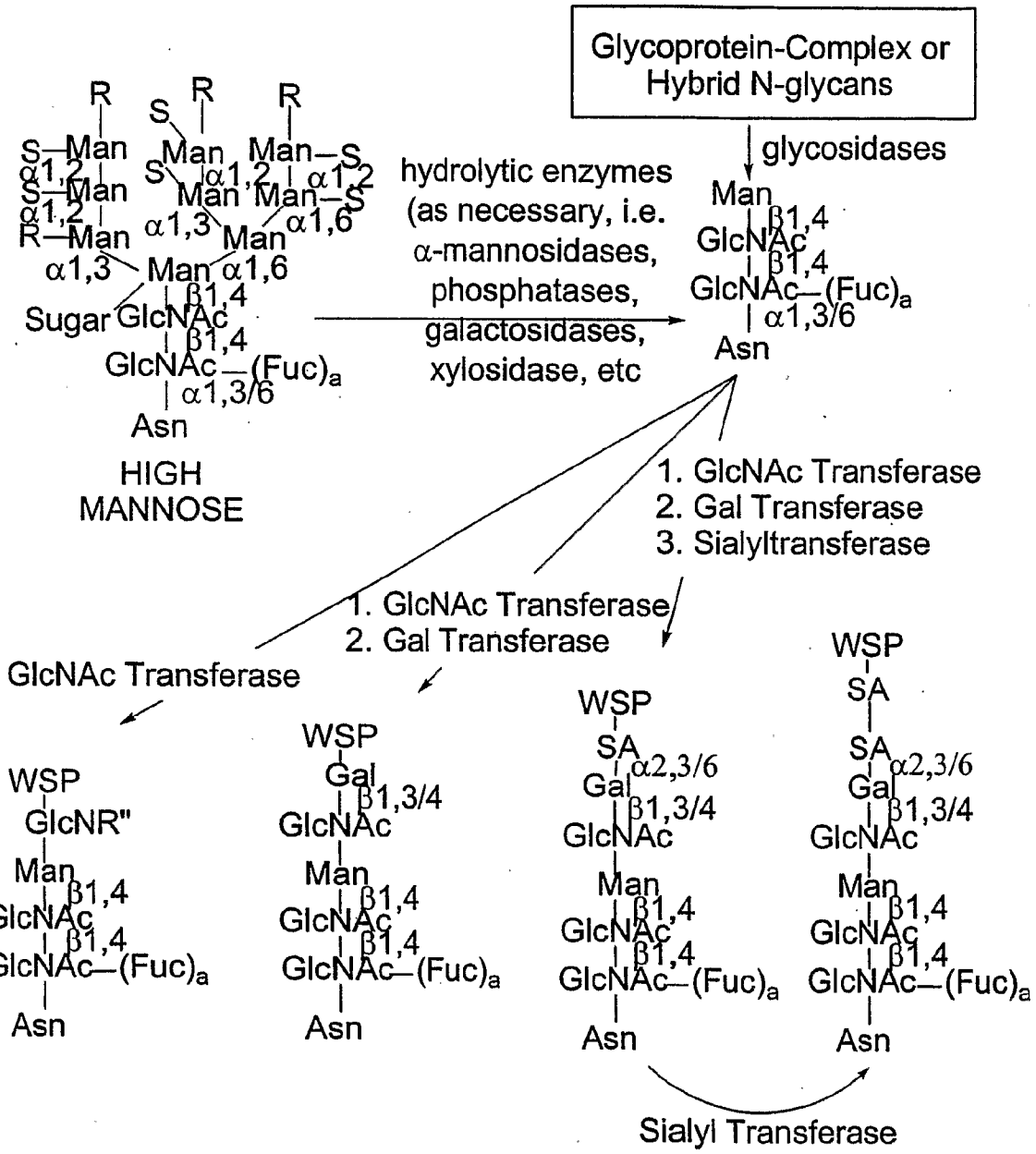

Glycoprotein-Complex or Hybrid N-glycans
glycosidases
hydrolytic enzymes (as necessary, i.e. α-mannosidases, phosphatases, galactosidases, xylosidase, etc
HIGH MANNOSE
1. GlcNAc Transferase
2. Gal Transferase
3. Sialyltransferase
1. GlcNAc Transferase
2. Gal Transferase
GlcNAc Transferase
Sialyl Transferase
WSP
GlcNR"
Gal
SA
Man
GlcNAc
Asn
Sugar
α1,2
α1,3
α1,6
β1,4
β1,3/4
α2,3/6
α1,3/6
(Fuc)a

FIG. 15

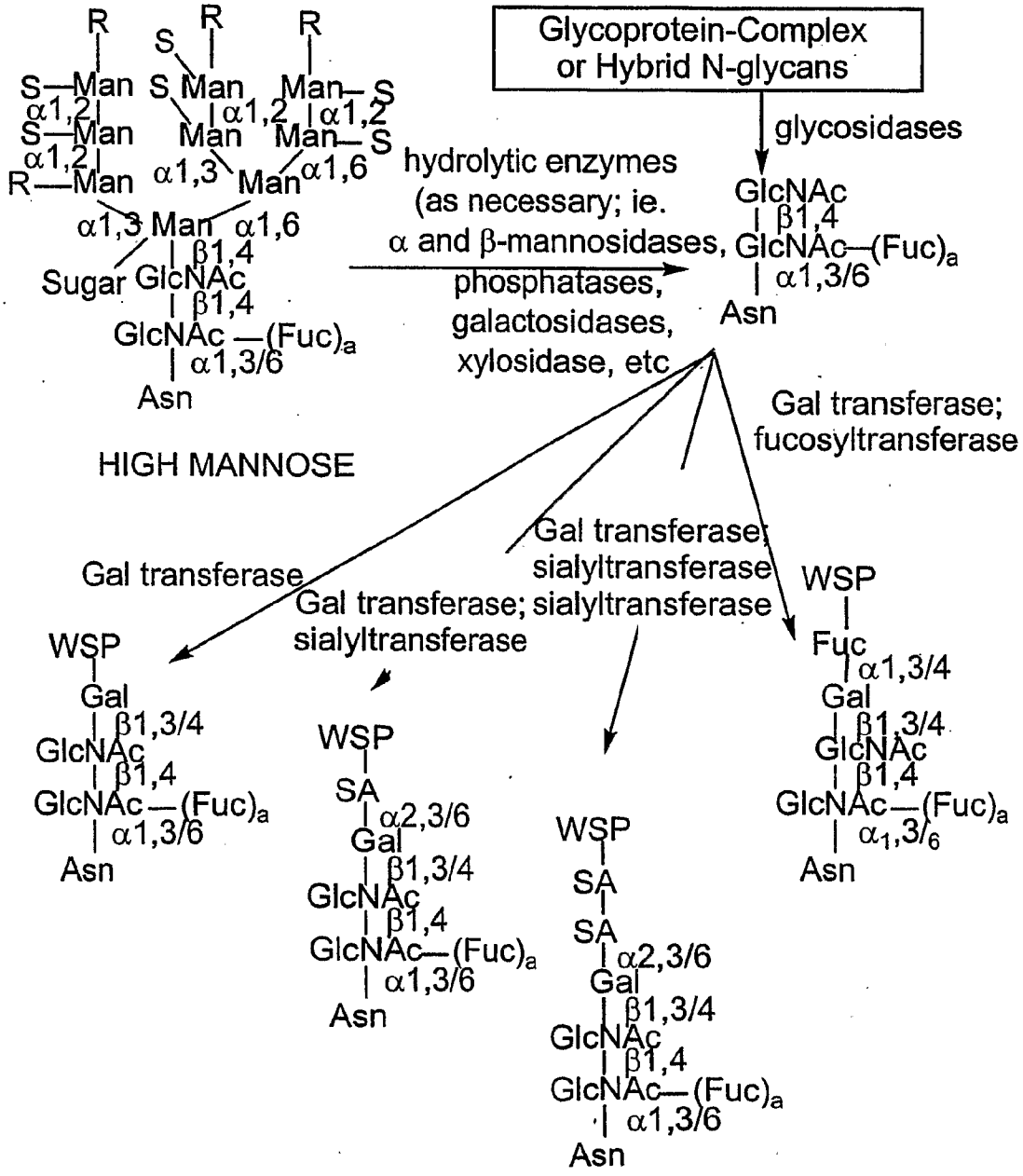

Glycoprotein-Complex or Hybrid N-glycans
glycosidases
hydrolytic enzymes (as necessary; ie. α and β-mannosidases, phosphatases, galactosidases, xylosidase, etc
HIGH MANNOSE
Gal transferase; fucosyltransferase
Gal transferase
Gal transferase; sialyltransferase
Gal transferase; sialyltransferase
WSP
Gal
β1,3/4
GlcNAc
β1,4
GlcNAc—(Fuc)a
α1,3/6
Asn
SA
α2,3/6
Fuc
α1,3/4
α1,3/6

FIG. 16

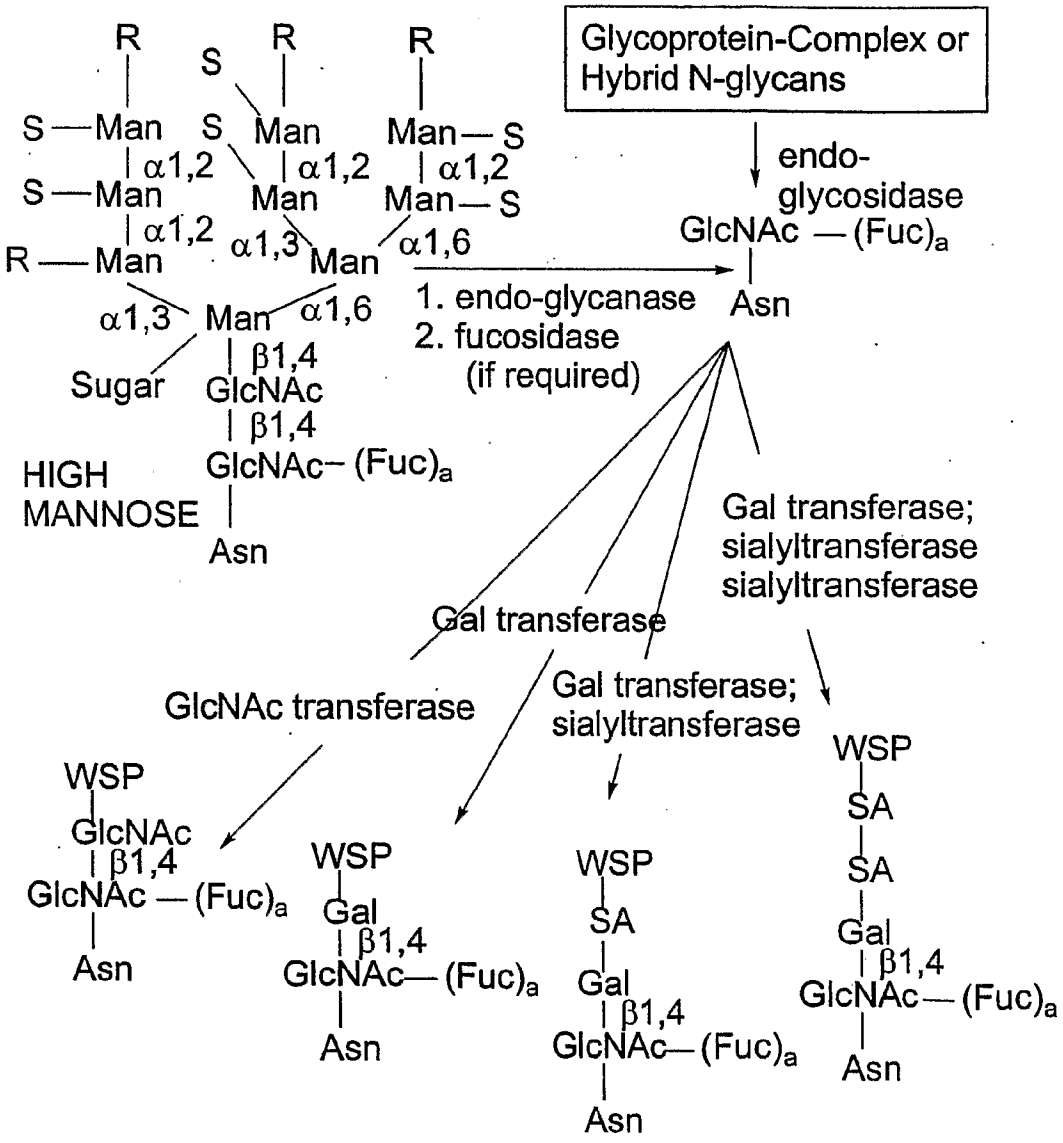

Glycoprotein-Complex or Hybrid N-glycans
endo-glycosidase
GlcNAc — (Fuc)a
Asn
1. endo-glycanase
2. fucosidase (if required)
HIGH MANNOSE
Sugar
Gal transferase
GlcNAc transferase
Gal transferase; sialyltransferase
Gal transferase; sialyltransferase
WSP
GlcNAc
β1,4
GlcNAc — (Fuc)a
Asn
Gal
SA

FIG. 17

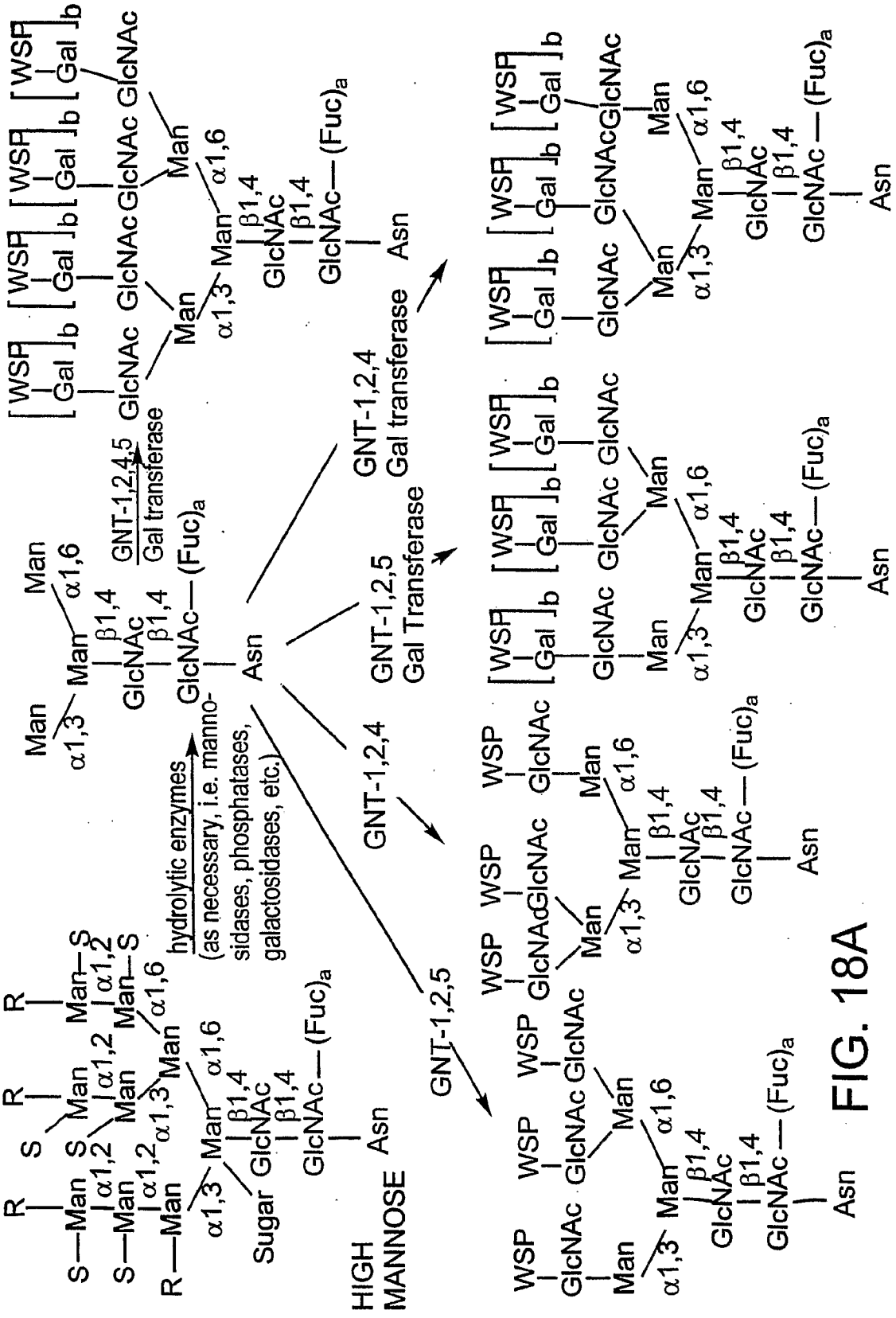

HIGH MANNOSE
hydrolytic enzymes (as necessary, i.e. mannosidases, phosphatases, galactosidases, etc.)
GNT-1,2,4,5 Gal transferase
GNT-1,2,4 Gal transferase
GNT-1,2,5 Gal Transferase
GNT-1,2,4
GNT-1,2,5
Asn
WSP
Gal
GlcNAc
Man
Sugar
α1,2
α1,3
α1,6
β1,4

FIG. 18A

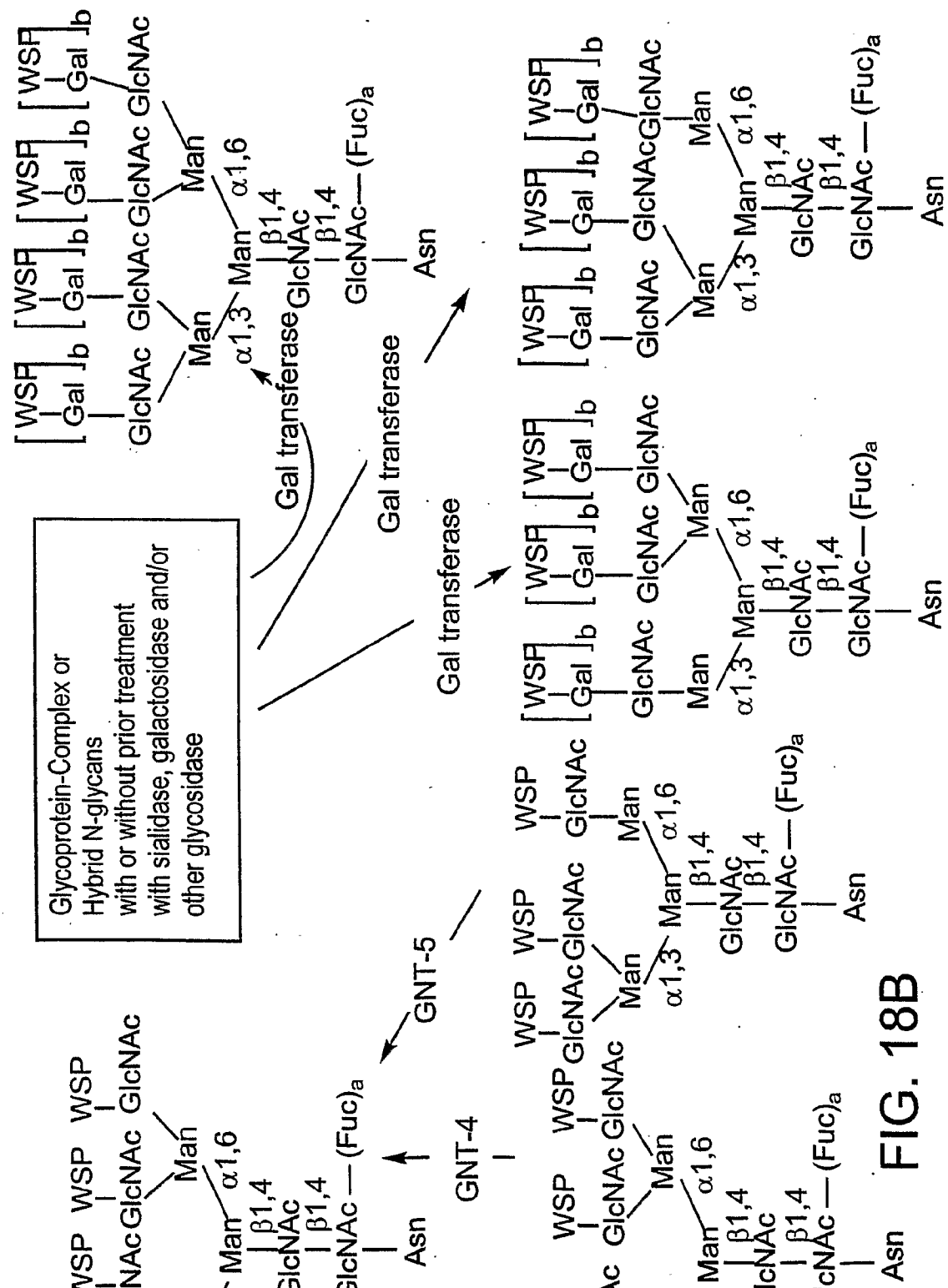

Glycoprotein-Complex or
Hybrid N-glycans
with or without prior treatment
with sialidase, galactosidase and/or
other glycosidase
Gal transferase
Gal transferase
Gal transferase
GNT-5
GNT-4
WSP
Gal]b
GlcNAc
Man
α1,3
α1,6
β1,4
Asn
(Fuc)a

FIG. 18B

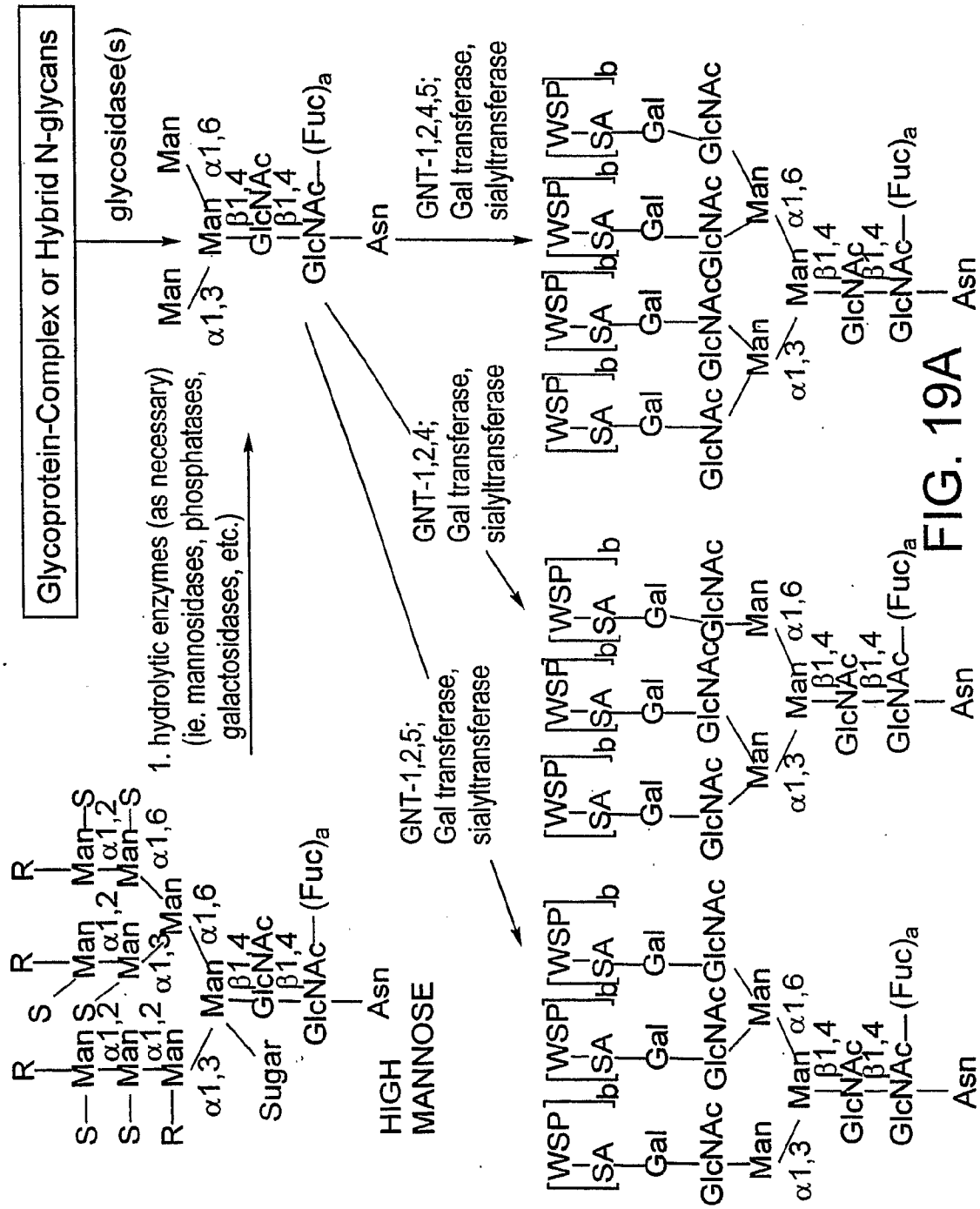

Glycoprotein-Complex or Hybrid N-glycans
glycosidase(s)
1. hydrolytic enzymes (as necessary) (ie. mannosidases, phosphatases, galactosidases, etc.)
HIGH MANNOSE
GNT-1,2,5; Gal transferase, sialyltransferase
GNT-1,2,4; Gal transferase, sialyltransferase
GNT-1,2,4,5; Gal transferase, sialyltransferase
Man
GlcNAc
Gal
Sugar
Asn
WSP
SA
Fuc

FIG. 19A

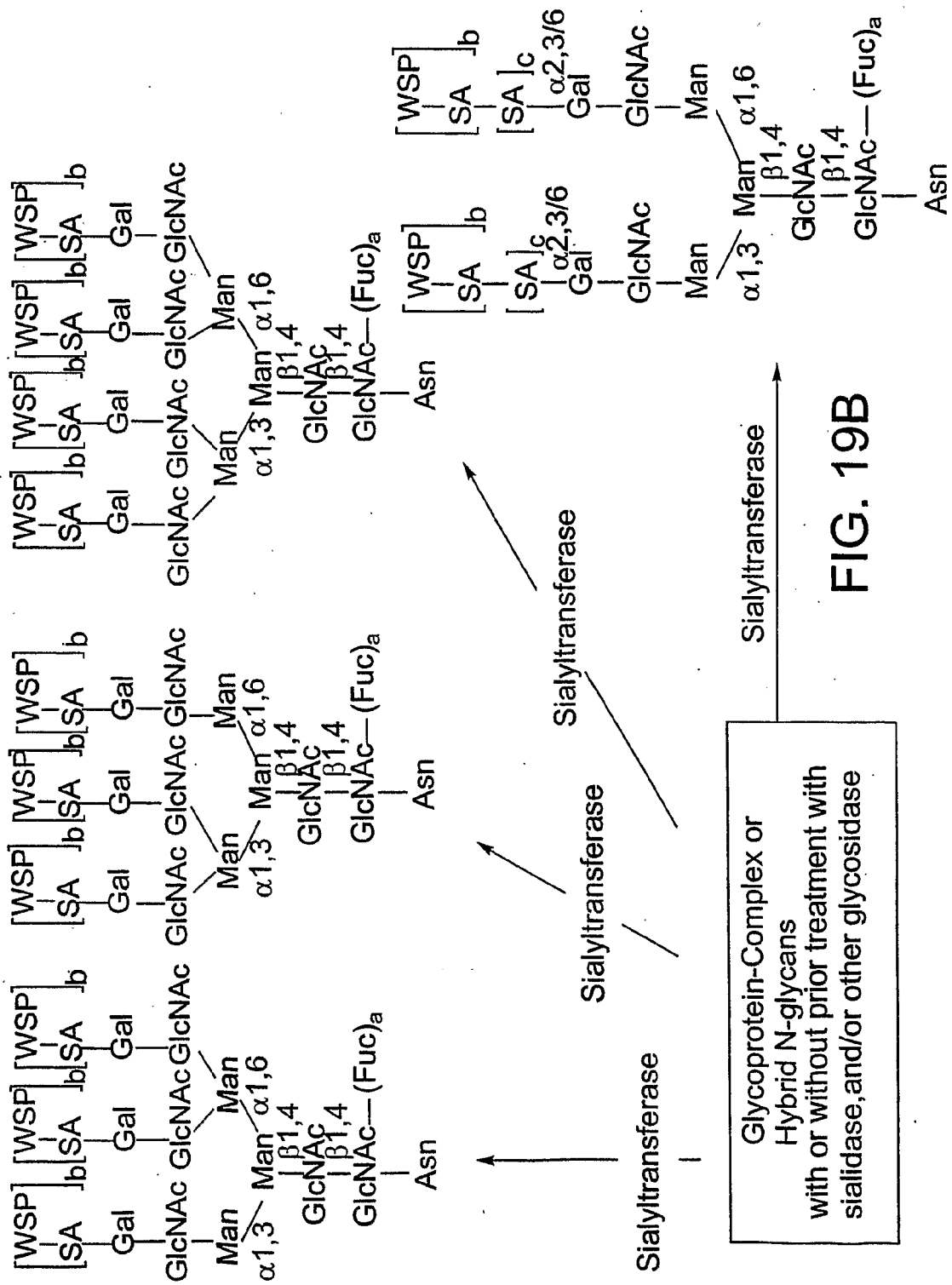

Glycoprotein-Complex or
Hybrid N-glycans
with or without prior treatment with
sialidase,and/or other glycosidase
Sialyltransferase
Sialyltransferase
Sialyltransferase
Sialyltransferase
[WSP]
SA
]b
[SA]c
Gal
GlcNAc
Man
α1,3
α1,6
α2,3/6
β1,4
Asn
(Fuc)a

FIG. 19B

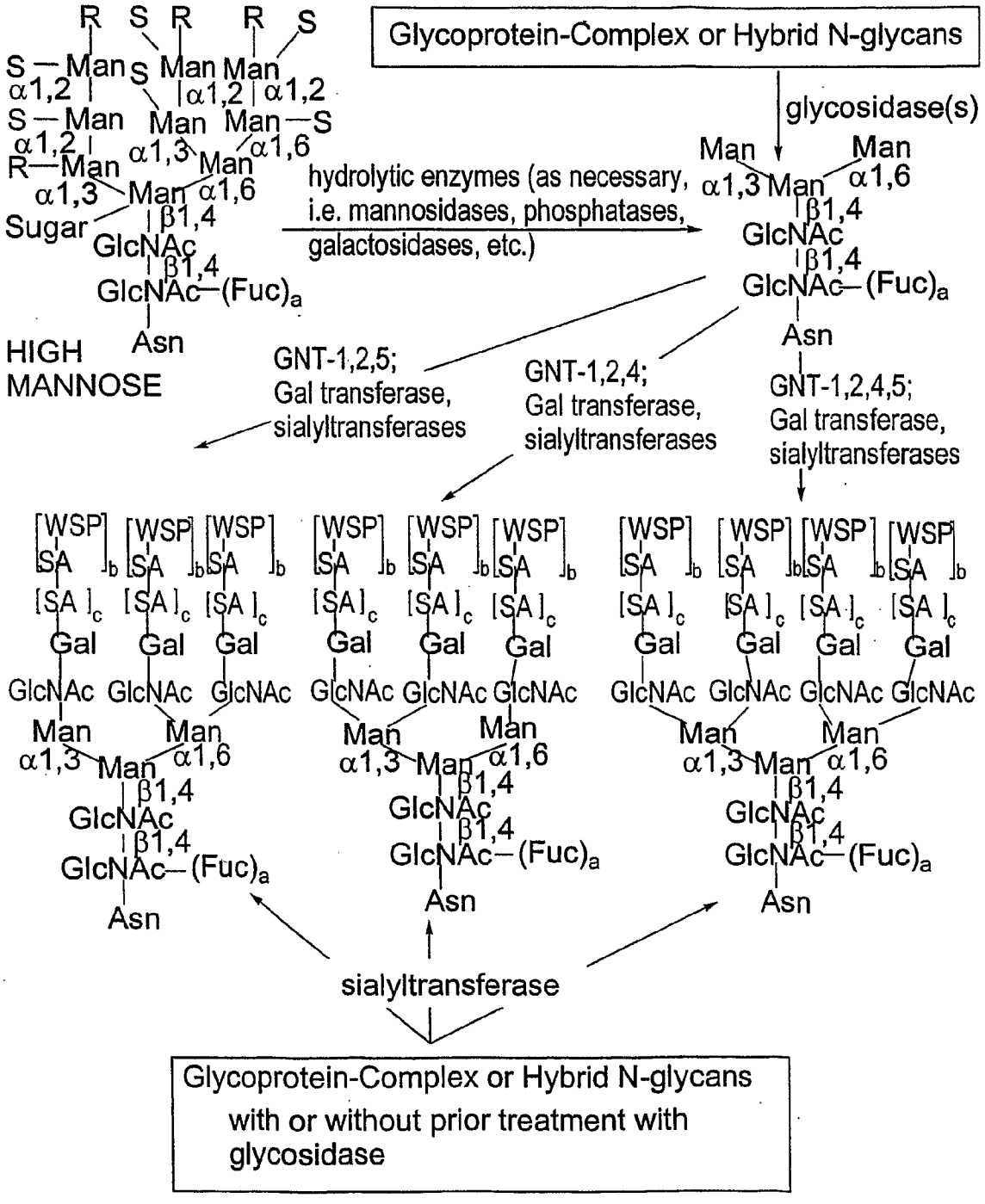

Glycoprotein-Complex or Hybrid N-glycans
glycosidase(s)
hydrolytic enzymes (as necessary, i.e. mannosidases, phosphatases, galactosidases, etc.)
HIGH MANNOSE
GNT-1,2,5; Gal transferase, sialyltransferases
GNT-1,2,4; Gal transferase, sialyltransferases
GNT-1,2,4,5; Gal transferase, sialyltransferases
sialyltransferase
Glycoprotein-Complex or Hybrid N-glycans with or without prior treatment with glycosidase

FIG. 20

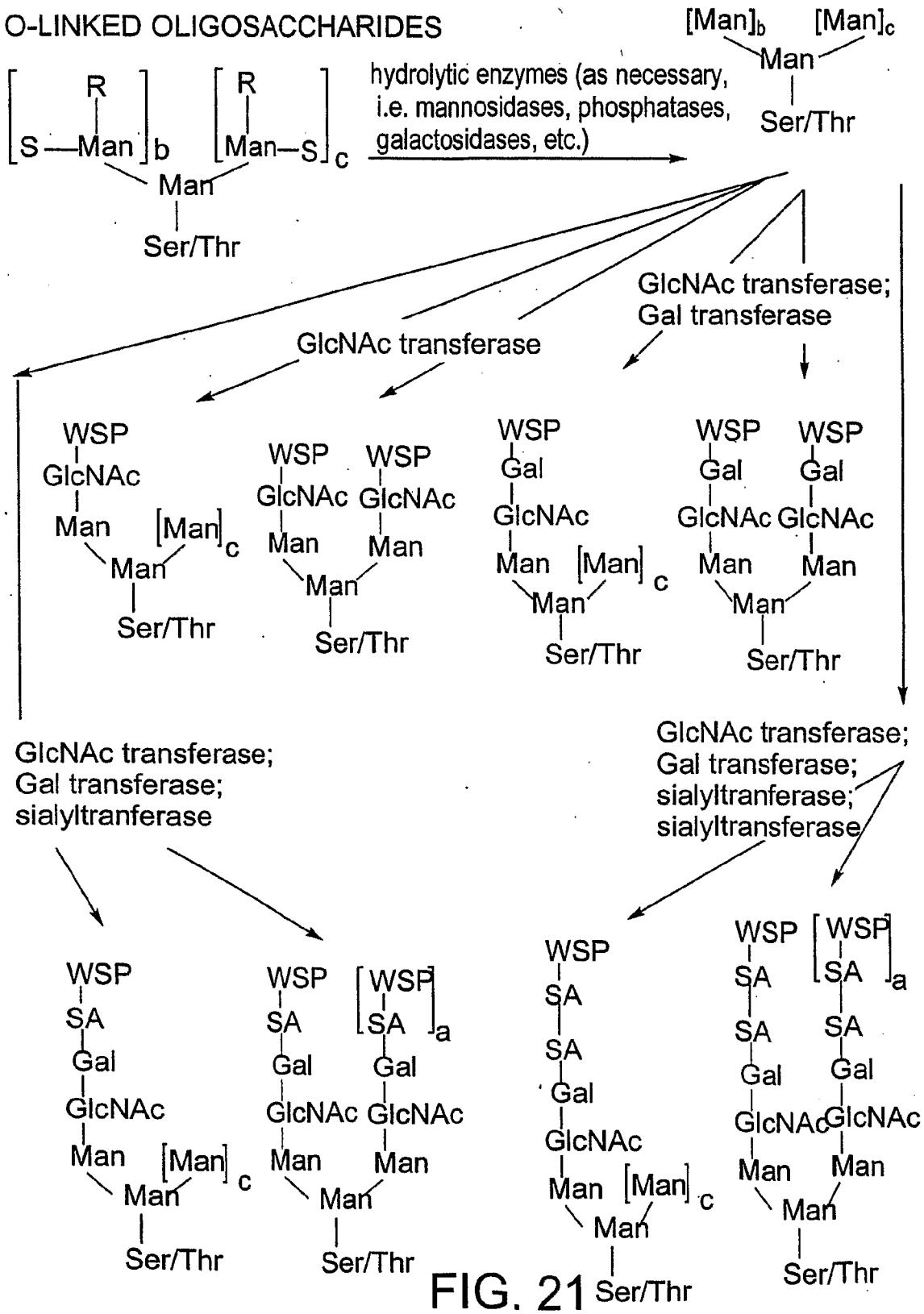

O-LINKED OLIGOSACCHARIDES
hydrolytic enzymes (as necessary, i.e. mannosidases, phosphatases, galactosidases, etc.)
Ser/Thr
GlcNAc transferase
GlcNAc transferase; Gal transferase
GlcNAc transferase; Gal transferase; sialyltranferase
GlcNAc transferase; Gal transferase; sialyltransferase
WSP
SA
Gal
GlcNAc
Man
Ser/Thr

FIG. 21

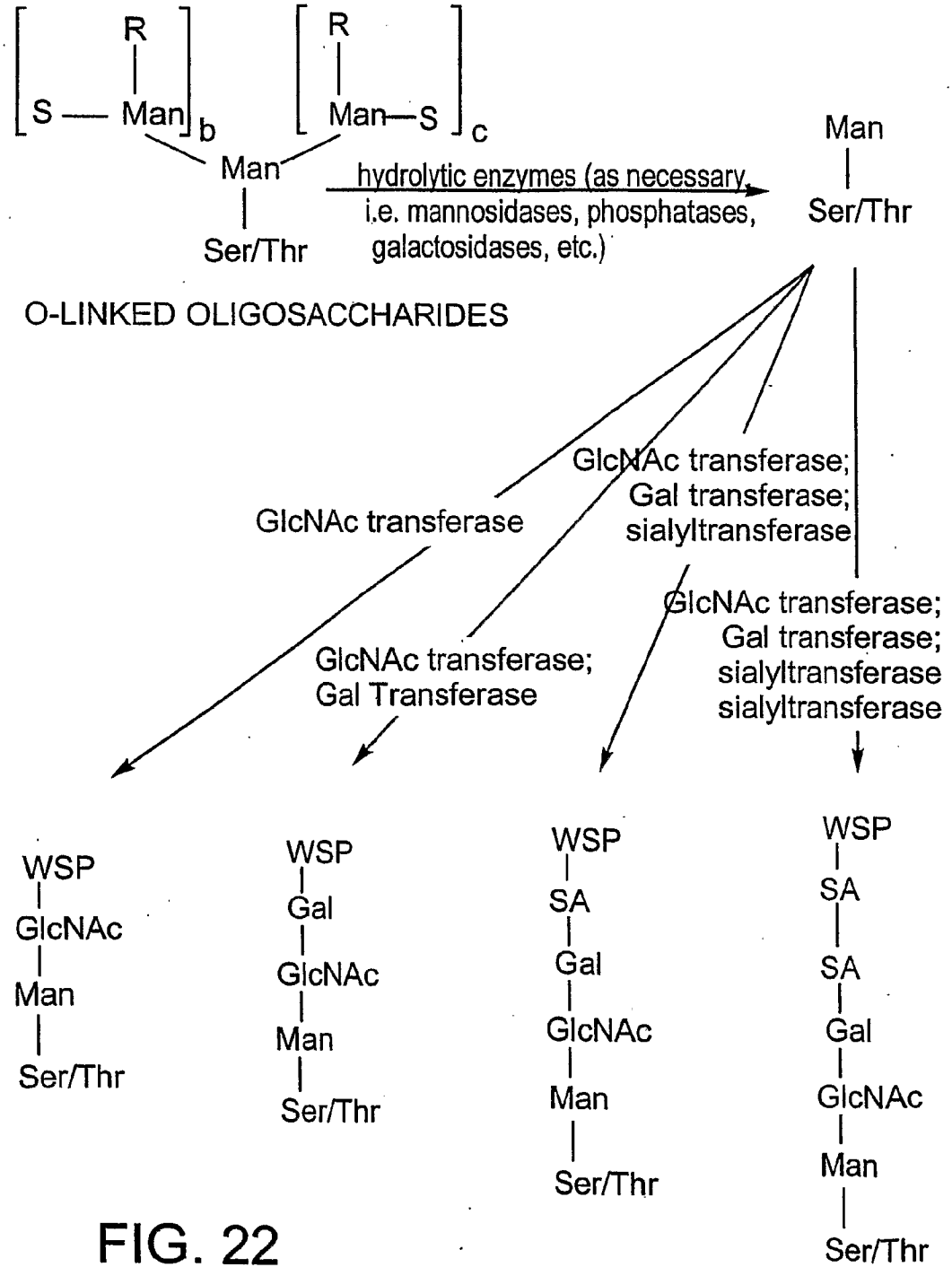

R
S — Man b
R
Man—S c
Man
Ser/Thr
hydrolytic enzymes (as necessary, i.e. mannosidases, phosphatases, galactosidases, etc.)
Man
Ser/Thr
O-LINKED OLIGOSACCHARIDES
GlcNAc transferase
GlcNAc transferase; Gal transferase; sialyltransferase
GlcNAc transferase; Gal Transferase
GlcNAc transferase; Gal transferase; sialyltransferase sialyltransferase
WSP
GlcNAc
Man
Ser/Thr
WSP
Gal
GlcNAc
Man
Ser/Thr
WSP
SA
Gal
GlcNAc
Man
Ser/Thr
WSP
SA
SA
Gal
GlcNAc
Man
Ser/Thr

FIG. 22

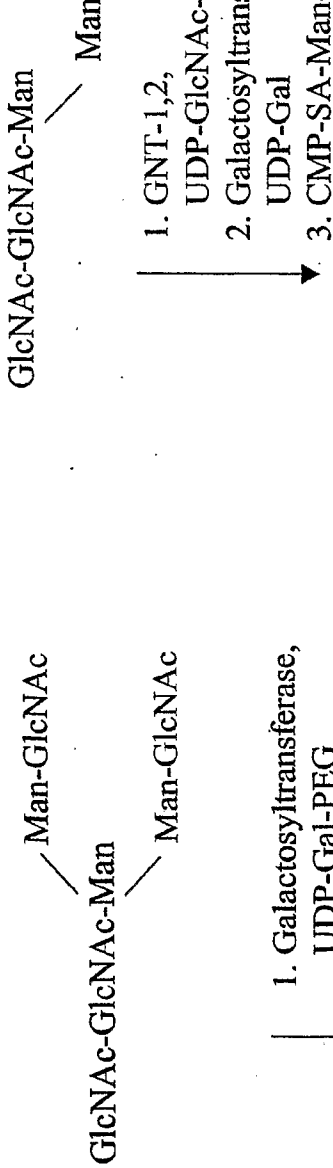


FIG. 23A

FIG. 23B

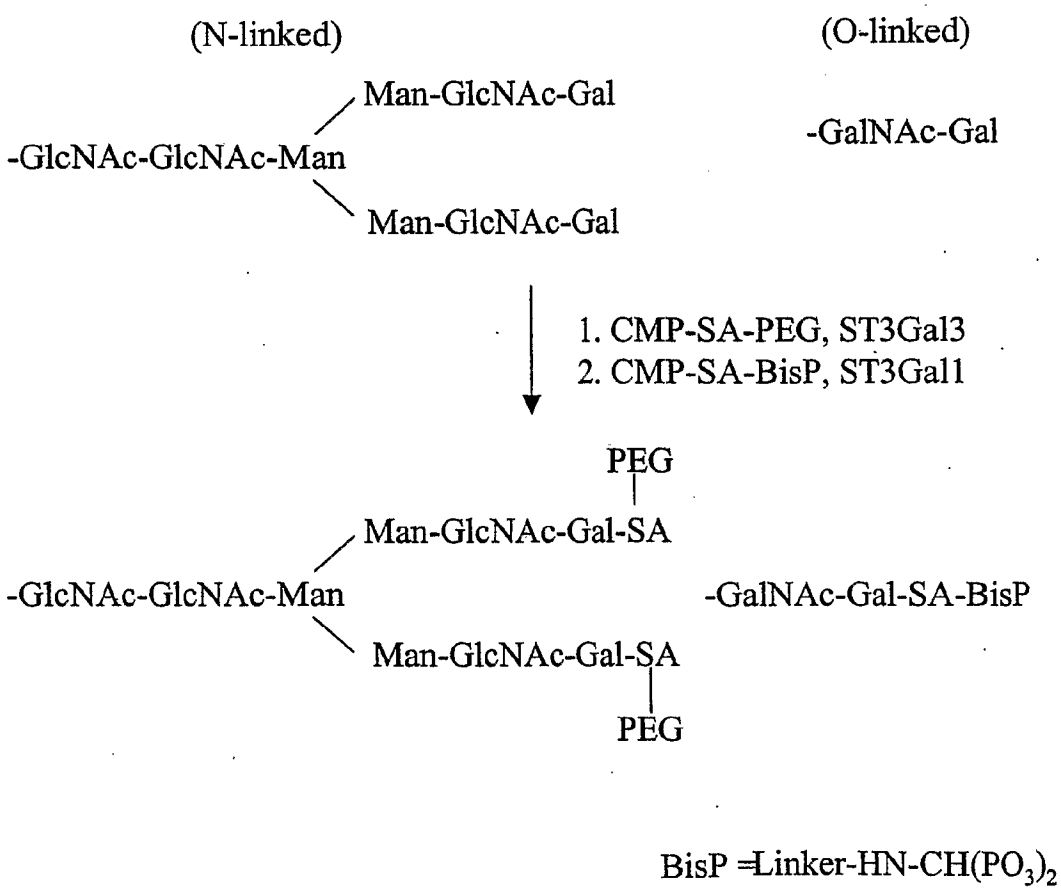

(N-linked)
(O-linked)
Man-GlcNAc-Gal
-GlcNAc-GlcNAc-Man
-GalNAc-Gal
Man-GlcNAc-Gal
1. CMP-SA-PEG, ST3Gal3
2. CMP-SA-BisP, ST3Gal1
PEG
Man-GlcNAc-Gal-SA
-GlcNAc-GlcNAc-Man
-GalNAc-Gal-SA-BisP
Man-GlcNAc-Gal-SA
PEG
BisP =Linker-HN-CH(PO3)2

FIG. 23C

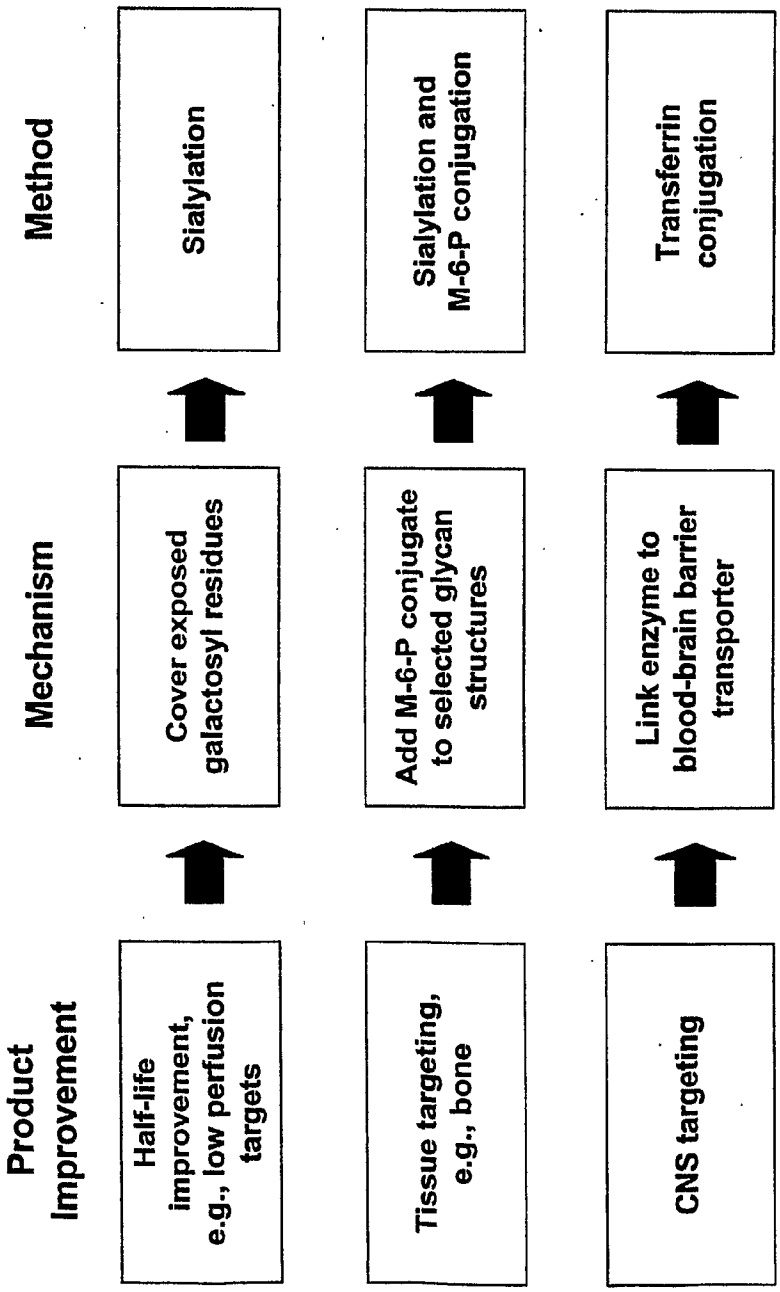

Product Improvement
Mechanism
Method
Half-life improvement, e.g., low perfusion targets
Cover exposed galactosyl residues
Sialylation
Tissue targeting, e.g., bone
Add M-6-P conjugate to selected glycan structures
Sialylation and M-6-P conjugation
CNS targeting
Link enzyme to blood-brain barrier transporter
Transferrin conjugation

FIG. 24

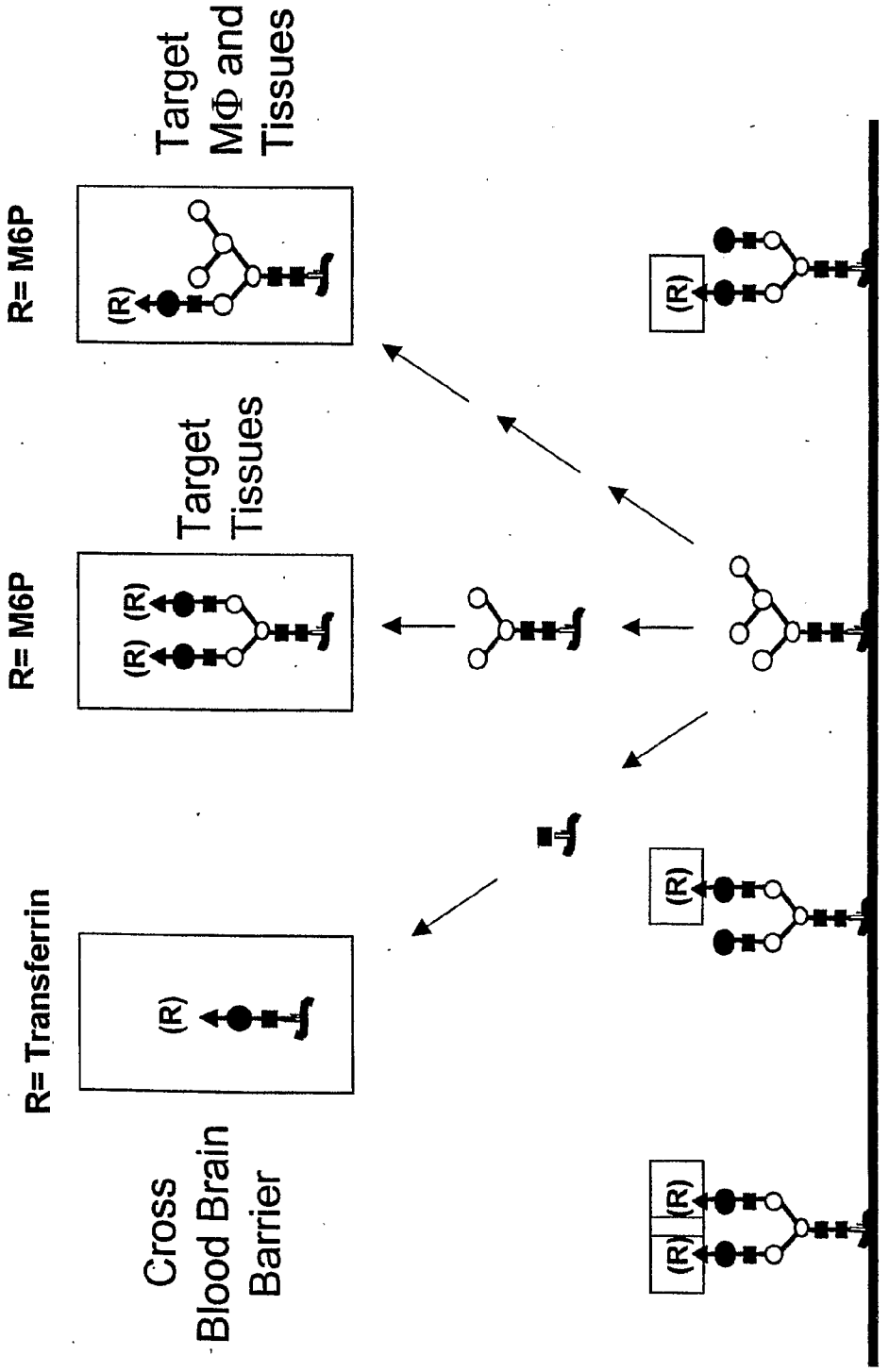

Target MΦ and Tissues
R= M6P
(R)
Target Tissues
R= M6P
(R)
(R)
R= Transferrin
(R)
Cross Blood Brain Barrier
(R)
(R)
(R)
(R)
(R)

FIG. 25

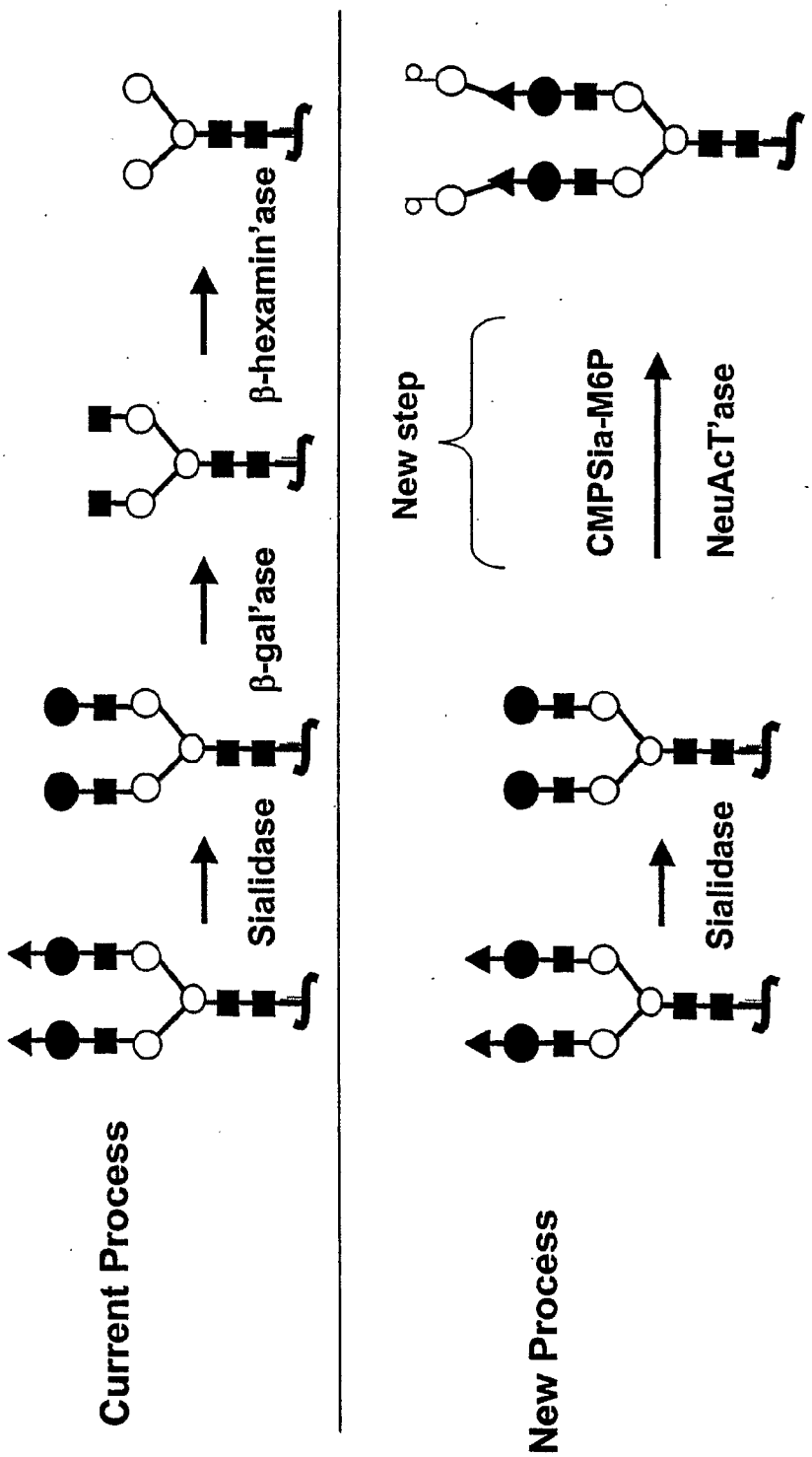

Current Process
Sialidase
β-gal'ase
β-hexamin'ase
New Process
Sialidase
New step
CMPSia-M6P
NeuAcT'ase

FIG. 26

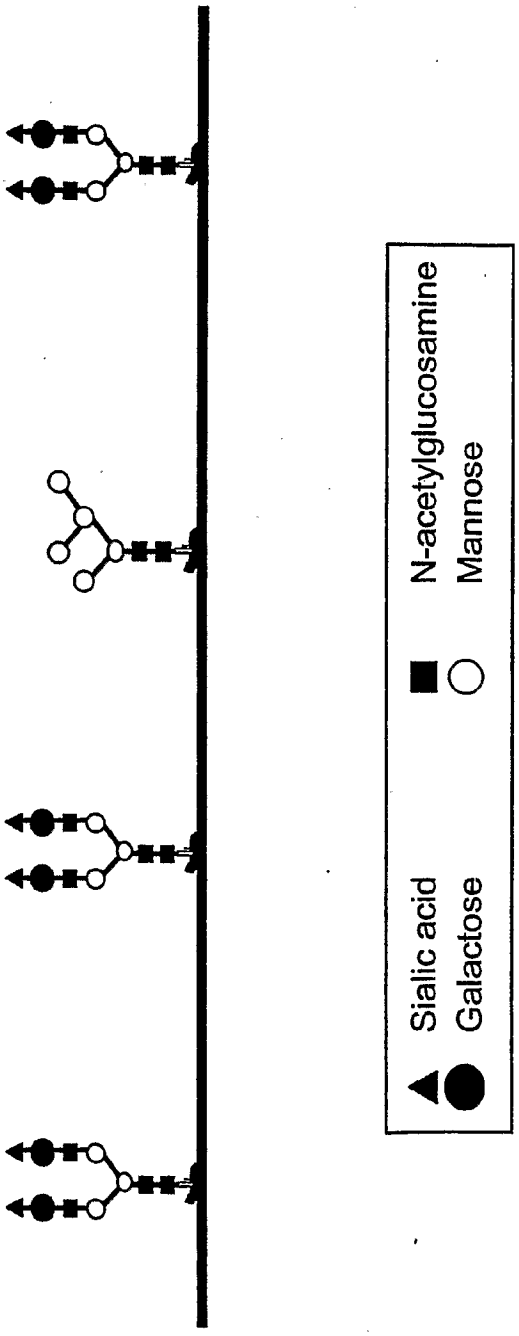


FIG. 27

12AP1/E5 -- Viventia Biotech
1964 -- Aventis
20K growth hormone -- AMUR
28P6/E6 -- Viventia Biotech
3-Hydroxyphthaloyl-beta-lactoglobulin –
4-IBB ligand gene therapy –
64-Cu MAb conjugate TETA-1A3 -- Mallinckrodt Institute of Radiology
64-Cu MAb conjugate TETA-cT84.66
64-Cu Trastuzumab TETA conjugate – Genentech
A 200 -- Amgen
A10255 – Eli Liliy
A1PDX – Hedral Therapeutics
A6 -- Angstrom
aaAT-III -- Genzyme
Abciximab -- Centocor
ABI.001 – Atlantic BioPharmaceuticals
ABT-828 – Abbott
Accutin
Actinohivin
activin -- Biotech Australia, Human Therapeutics, Curis
AD 439 – Tanox
AD 519 – Tanox
Adalimumab -- Cambridge Antibody Tech.
Adenocarcinoma vaccine – Biomira -- NIS
Adenosine deanimase -- Enzond
Adenosine A2B receptor antagonists -- Adenosine Therapeutics
ADP-001 – Axis Genetics
AF 13948 – Affymax
Afelimomab – Knoll
AFP-SCAN – Immunomedics
AG 2195 – Corixa
agalsidase alfa -- Transkaryotic Therapies
agalsidase beta -- Genzyme
AGENT– Antisoma
AI 300 – AutoImmune
AI-101 – Teva
AI-102 – Teva
AI-201 – AutoImmune
AI-301 – AutoImmune
AIDS vaccine – ANRS, CIBG, Hesed Biomed, Hollis-Eden, Rome, United Biomedical, American Home Products, Maxygen
airway receptor ligand -- IC Innovations
AJvW 2 -- Ajinomoto
AK 30 NGF -- Alkermes
Albuferon -- Human Genome Sciences
albumin – Biogen, DSM Anti-Infectives, Genzyme Transgenics, PPL Therapeutics, TranXenoGen, Welfide Corp.
aldesleukin -- Chiron
alefacept -- Biogen
Alemtuzumab
Allergy therapy -- ALK-Abello/Maxygen, ALK-Abello/RP Scherer
allergy vaccines -- Allergy Therapeutics
Alnidofibatide -- Aventis Pasteur
Alnorine -- SRC VB VECTOR
ALP 242 -- Gruenenthal
Alpha antitrypsin -- Arriva/Hyland Immuno/ProMetic/Protease Sciences
Alpha-1 antitrypsin – Cutter, Bayer, PPL Therapeutics, Profile, ZymoGenetics, Arriva
Alpha-1 protease inhibitor -- Genzyme Transgenics, Welfide Corp.
Alpha-galactose fusion protein – Immunomedics
Alpha-galactosidase A -- Research Corporation Technologies, Genzyme
Alpha-glucosidase – Genzyme, Novazyme
Alpha-lactalbumin
Alpha-L-iduronidase -- Transkaryotic Therapies, BioMarin
alteplase -- Genentech
alvircept sudotox – NIH
ALX-0600, a GLP-2 agonist -- NPS Allelix Corp.

FIG. 28A

ALX1-11 –sNPS Pharmaceuticals
Alzheimer's disease gene therapy
AM-133 -- AMRAD
Amb a 1 immunostim conj. -- Dynavax
AMD 3100 – AnorMED -- NIS
AMD 3465 – AnorMED -- NIS
AMD 3465 – AnorMED -- NIS
AMD Fab -- Genentech
Amediplase – Menarini, Novartis
AM-F9
Amoebiasis vaccine
Amphiregulin -- Octagene
anakinra -- Amgen
analgesic -- Nobex
ancestim -- Amgen
AnergiX.RA – Corixa, Organon
Angiocidin -- InKine
angiogenesis inhibitors -- ILEX
AngioMab – Antisoma
Angiopoietins -- Regeneron/Procter & Gamble
angiostatin -- EntreMed
Angiostatin/endostatin gene therapy -- Genetix Pharmaceuticals
angiotensin-II, topical -- Maret
Anthrax -- EluSys Therapeutics/US Army Medical Research Institute
Anthrax vaccine
Anti platelet-derived growth factor D human monoclonal antibodies -- CuraGen
Anti-17-1A MAb 3622W94 -- GlaxoSmithKline
Anti-2C4 MAb -- Genentech
anti-4-1BB monoclonal antibodies -- Bristol-Myers Squibb
Anti-Adhesion Platform Tech. -- Cytovax
Anti-adipocyte MAb -- Cambridge Antibody Tech./ObeSys
antiallergics -- Maxygen
antiallergy vaccine -- Acambis
Anti-alpha-4-integrin MAb
Anti-alphavβ3 integrin MAb – Applied Molecular Evolution
Anti-angiogenesis monoclonal antibodies -- KS Biomedix/Schering AG
Anti-B4 MAb-DC1 conjugate -- ImmunoGen
Anti-B7 antibody PRIMATIZED -- IDEC
Anti-B7-1 MAb 16-10A1
Anti-B7-1 MAb 1G10
Anti-B7-2 MAb GL-1
Anti-B7-2-gelonin immunotoxin –
Antibacterials/antifungals -- Diversa/IntraBiotics
Anti-beta-amyloid monoclonal antibodies -- Cambridge Antibody Tech., Wyeth-Ayerst
Anti-BLyS antibodies -- Cambridge Antibody Tech. /Human Genome Sciences
Antibody-drug conjugates -- Seattle Genetics/Eos
Anti-C5 MAb BB5-1 -- Alexion
Anti-C5 MAb N19-8 -- Alexion
Anti-C8 MAb
anticancer cytokines -- BioPulse
anticancer matrix – Telios Integra
Anticancer monoclonal antibodies – ARIUS, Immunex
anticancer peptides – Maxygen, Micrologix
Anticancer prodrug Tech. -- Alexion Antibody Technologies
anticancer Troy-Bodies -- Affite -- Affitech
anticancer vaccine -- NIH
anticancers -- Epimmune
Anti-CCR5/CXCR4 sheep MAb -- KS Biomedix Holdings
Anti-CD11a MAb KBA –
Anti-CD11a MAb M17
Anti-CD11a MAb TA-3 –
Anti-CD11a MAb WT.1 –
Anti-CD11b MAb -- Pharmacia
Anti-CD11b MAb LM2
Anti-CD154 MAb -- Biogen
Anti-CD16-anti-CD30 MAb -- Biotest

FIG. 28B

Anti-CD18 MAb -- Pharmacia
Anti-CD19 MAb B43 –
Anti-CD19 MAb -liposomal sodium butyrate conjugate –
Anti-CD147
Anti-CD19 MAb-saporin conjugate –
Anti-CD19-dsFv-PE38-immunotoxin –
Anti-CD2 MAb 12-15 –
Anti-CD2 MAb B-E2 -- Diaclone
Anti-CD2 MAb OX34 –
Anti-CD2 MAb OX54 –
Anti-CD2 MAb OX55 –
Anti-CD2 MAb RM2-1
Anti-CD2 MAb RM2-2
Anti-CD2 MAb RM2-4
Anti-CD20 MAb BCA B20
Anti-CD20-anti-Fc alpha RI bispecific MAb – Medarex, Tenovus
Anti-CD22 MAb-saporin-6 complex –
Anti-CD3 immunotoxin –
Anti-CD3 MAb 145-2C11 -- Pharming
Anti-CD3 MAb CD4IgG conjugate -- Genentech
Anti-CD3 MAb humanised – Protein Design, RW Johnson
Anti-CD3 MAb WT32
Anti-CD3 MAb-ricin-chain-A conjugate –
Anti-CD3 MAb-xanthine-oxidase conjugate –
Anti-CD30 MAb BerH2 -- Medac
Anti-CD30 MAb-saporin conjugate
Anti-CD30-scFv-ETA'-immunotoxin
Anti-CD38 MAb AT13/5
Anti-CD38 MAb-saporin conjugate
Anti-CD3-anti-CD19 bispecific MAb
Anti-CD3-anti-EGFR MAb
Anti-CD3-anti-interleukin-2-receptor MAb
Anti-CD3-anti-MOv18 MAb -- Centocor
Anti-CD3-anti-SCLC bispecific MAb
Anti-CD4 idiotype vaccine
Anti-CD4 MAb – Centocor, IDEC Pharmaceuticals, Xenova Group
Anti-CD4 MAb 16H5
Anti-CD4 MAb 4162W94 – GlaxoSmithKline
Anti-CD4 MAb B-F5 -- Diaclone
Anti-CD4 MAb GK1-5
Anti-CD4 MAb KT6
Anti-CD4 MAb OX38
Anti-CD4 MAb PAP conjugate -- Bristol-Myers Squibb
Anti-CD4 MAb RIB 5-2
Anti-CD4 MAb W3/25
Anti-CD4 MAb YTA 3.1.2
Anti-CD4 MAb YTS 177-9
Anti-CD40 ligand MAb 5c8 -- Biogen
Anti-CD40 MAb
Anti-CD40 MAb 5D12 – Tanox
Anti-CD44 MAb A3D8
Anti-CD44 MAb GKWA3
Anti-CD44 MAb IM7
Anti-CD44 MAb KM81
Anti-CD44 variant monoclonal antibodies -- Corixa/Hebrew University
Anti-CD45 MAb BC8-I-131
Anti-CD45RB MAb
Anti-CD48 MAb HuLy-m3
Anti-CD48 MAb WM-63
Anti-CD5 MAb -- Becton Dickinson
Anti-CD5 MAb OX19
Anti-CD6 MAb
Anti-CD7 MAb-PAP conjugate
Anti-CD7 MAb-ricin-chain-A conjugate
Anti-CD8 MAb – Amerimmune, Cytodyn, Becton Dickinson
Anti-CD8 MAb 2-43
Anti-CD8 MAb OX8
Anti-CD80 MAb P16C10 -- IDEC
Anti-CD80 MAb P7C10 -- ID Vaccine
Anti-CD8-idarubicin conjugate
Anti-CEA MAb CE-25
Anti-CEA MAb MN 14 – Immunomedics

FIG. 28C

Anti-CEA MAb MN14-PE40 conjugate – Immunomedics
Anti-CEA MAb T84.66-interleukin-2 conjugate
Anti-CEA sheep MAb -- KS Biomedix Holdings
Anti-cell surface monoclonal antibodies -- Cambridge Antibody Tech. /Pharmacia
Anti-c-erbB2-anti-CD3 bifunctional MAb -- Otsuka
Anti-CMV MAb -- Scotgen
Anti-complement
Anti-CTLA-4 MAb
Anti-EGFR catalytic antibody -- Hesed Biomed
anti-EGFR immunotoxin -- IVAX
Anti-EGFR MAb -- Abgenix
Anti-EGFR MAb 528
Anti-EGFR MAb KSB 107 -- KS Biomedix
Anti-EGFR MAb-DM1 conjugate -- ImmunoGen
Anti-EGFR MAb-LA1 –
Anti-EGFR sheep MAb -- KS Biomedix
Anti-FAP MAb F19-I-131
Anti-Fas IgM MAb CH11
Anti-Fas MAb Jo2
Anti-Fas MAb RK-8
Anti-Flt-1 monoclonal antibodies -- ImClone
Anti-fungal peptides -- State University of New York
antifungal tripeptides -- BTG
Anti-ganglioside GD2 antibody-interleukin-2 fusion protein -- Lexigen
Anti-GM2 MAb -- Kyowa
Anti-GM-CSF receptor monoclonal antibodies -- AMRAD
Anti-gp130 MAb -- Tosoh
Anti-HCA monoclonal antibodies -- AltaRex/Epigen
Anti-hCG antibodies -- Abgenix/AVI BioPharma
Anti-heparanase human monoclonal antibodies -- Oxford Glycosciences/Medarex
Anti-hepatitis C virus human monoclonal antibodies -- XTL Biopharmaceuticals
Anti-HER-2 antibody gene therapy
Anti-herpes antibody -- Epicyte
Anti-HIV antibody -- Epicyte
anti-HIV catalytic antibody -- Hesed Biomed
anti-HIV fusion protein -- Idun
anti-HIV proteins -- Cangene
Anti-HM1-24 MAb -- Chugai
Anti-hR3 MAb
Anti-Human-Carcinoma-Antigen MAb -- Epicyte
Anti-ICAM-1 MAb -- Boehringer Ingelheim
Anti-ICAM-1 MAb 1A-29 -- Pharmacia
Anti-ICAM-1 MAb HA58
Anti-ICAM-1 MAb YN1/1.7.4
Anti-ICAM-3 MAb ICM3 -- ICOS
Anti-idiotype breast cancer vaccine 11D10
Anti-idiotype breast cancer vaccine ACA14C5 –
Anti-idiotype cancer vaccine -- ImClone Systems/Merck KGaA ImClone, Viventia Biotech
Anti-idiotype cancer vaccine 1A7 -- Titan
Anti-idiotype cancer vaccine 3H1 -- Titan
Anti-idiotype cancer vaccine TriAb -- Titan
Anti-idiotype Chlamydia trachomatis vaccine
Anti-idiotype colorectal cancer vaccine -- Novartis
Anti-idiotype colorectal cancer vaccine -- Onyvax
Anti-idiotype melanoma vaccine -- IDEC Pharmaceuticals
Anti-idiotype ovarian cancer vaccine ACA 125
Anti-idiotype ovarian cancer vaccine AR54 -- AltaRex

FIG. 28D

Anti-idiotype ovarian cancer vaccine CA-125 – AltaRex, Biomira
Anti-IgE catalytic antibody -- Hesed Biomed
Anti-IgE MAb E26 -- Genentech
Anti-IGF-1 MAb
anti-inflammatory -- GeneMax
anti-inflammatory peptide -- BTG
anti-integrin peptides -- Burnha
Anti-interferon-alpha-receptor MAb 64G12 -- Pharma Pacific Management
Anti-interferon-gamma MAb -- Protein Design Labs
Anti-interferon-gamma polyclonal antibody -- Advanced Biotherapy
Anti-interleukin-10 MAb –
Anti-interleukin-12 MAb –
Anti-interleukin-1-beta polyclonal antibody -- R&D Systems
Anti-interleukin-2 receptor MAb 2A3
Anti-interleukin-2 receptor MAb 33B3-1 -- Immunotech
Anti-interleukin-2 receptor MAb ART-18
Anti-interleukin-2 receptor MAb LO-Tact-1
Anti-interleukin-2 receptor MAb Mikbeta1
Anti-interleukin-2 receptor MAb NDS61
Anti-interleukin-4 MAb 11B11
Anti-interleukin-5 MAb -- Wallace Laboratories
Anti-interleukin-6 MAb – Centocor, Diaclone, Pharmadigm
Anti-interleukin-8 MAb -- Abgenix
Anti-interleukin-8 MAb – Xenotech
Anti-JL1 MAb
Anti-Klebsiella sheep MAb -- KS Biomedix Holdings
Anti-Laminin receptor MAb-liposomal doxorubicin conjugate
Anti-LCG MAb -- Cytoclonal
Anti-lipopolysaccharide MAb -- VitaResc
Anti-L-selectin monoclonal antibodies -- Protein Design Labs, Abgenix, Stanford University
Anti-MBL monoclonal antibodies -- Alexion/Brigham and Women's Hospital
Anti-MHC monoclonal antibodies
Anti-MIF antibody humanised – IDEC, Cytokine PharmaSciences
Anti-MRSA/VRSA sheep MAb -- KS Biomedix Holdings
Anti-mu MAb -- Novartis
Anti-MUC-1 MAb
Anti-MUC 18
Anti-Nogo-A MAb IN1
Anti-nuclear autoantibodies -- Procyon
Anti-ovarian cancer monoclonal antibodies -- Dompe
Anti-p185 monoclonal antibodies
Anti-p43 MAb
Antiparasitic vaccines
Anti-PDGF/bFGF sheep MAb -- KS Biomedix
Anti-properdin monoclonal antibodies -- Abgenix/Gliatech
Anti-PSMA (prostrate specific membrane antigen)
Anti-PSMA MAb J591 -- BZL Biologics
Anti-Rev MAb gene therapy –
Anti-RSV antibodies – Epicyte, Intracell
Anti-RSV monoclonal antibodies -- Medarex/MedImmune, Applied Molecular Evolution/MedImmune
Anti-RSV MAb, inhalation -- Alkermes/MedImmune
Anti-RT gene therapy
Antisense K-ras RNA gene therapy
Anti-SF-25 MAb
Anti-sperm antibody -- Epicyte
Anti-Tac(Fv)-PE38 conjugate
Anti-TAPA/CD81 MAb AMP1
Anti-tat gene therapy

FIG. 28E

Anti-TCR-alphabeta MAb H57-597
Anti-TCR-alphabeta MAb R73
Anti-tenascin MAb BC-4-I-131
Anti-TGF-beta human monoclonal antibodies -- Cambridge Antibody Tech., Genzyme
Anti-TGF-beta MAb 2G7 -- Genentech
Antithrombin III -- Genzyme Transgenics, Aventis, Bayer, Behringwerke, CSL, Myriad
Anti-Thy1 MAb
Anti-Thy1.1 MAb
Anti-tissue factor/factor VIIA sheep MAb -- KS Biomedix
Anti-TNF monoclonal antibodies – Centocor, Chiron, Peptech, Pharacia, Serono
Anti-TNF sheep MAb -- KS Biomedix Holdings
Anti-TNFalpha MAb -- Genzyme
Anti-TNFalpha MAb B-C7 -- Diaclone
Anti-tooth decay MAb -- Planet BioTech.
Anti-TRAIL receptor-1 MAb -- Takeda
Antitumour RNases -- NIH
Anti-VCAM MAb 2A2 -- Alexion
Anti-VCAM MAb 3F4 -- Alexion
Anti-VCAM-1 MAb
Anti-VEC MAb -- ImClone
Anti-VEGF MAb -- Genentech
Anti-VEGF MAb 2C3
Anti-VEGF sheep MAb -- KS Biomedix Holdings
Anti-VLA-4 MAb HP1/2 -- Biogen
Anti-VLA-4 MAb PS/2
Anti-VLA-4 MAb R1-2
Anti-VLA-4 MAb TA-2
Anti-VAP-1 human MAb
Anti-VRE sheep MAb -- KS Biomedix Holdings
ANUP -- TranXenoGen
ANUP-1 -- Pharis
AOP-RANTES -- Senetek
Apan-CH -- Praecis Pharmaceuticals
APC-8024 -- Demegen
ApoA-1 -- Milano, Pharmacia
Apogen -- Alexion
apolipoprotein A1 -- Avanir
Apolipoprotein E -- Bio-Tech. General
Applaggin -- Biogen
aprotinin -- ProdiGene
APT-070C -- AdProTech
AR 177 -- Aronex Pharmaceuticals
AR 209 -- Aronex Pharmaceuticals, Antigenics
AR545C
ARGENT gene delivery systems -- ARIAD
Arresten
ART-123 -- Asahi Kasei
arylsulfatase B -- BioMarin
Arylsulfatase B, Recombinant human -- BioMarin
AS 1051 -- Ajinomoto
ASI-BCL -- Intracell
Asparaginase - Merck
ATL-101 -- Alizyme
Atrial natriuretic peptide -- Pharis
Aurintricarboxylic acid-high molecular weight
Autoimmune disorders -- GPC Biotech/MorphoSys
Autoimmune disorders and transplant rejection -- Bristol-Myers Squibb/Genzyme Tra
Autoimmune disorders/cancer -- Abgenix/Chiron, CuraGen
Autotaxin
Avicidin -- NeoRx
axogenesis factor-1 -- Boston Life Sciences
Axokine -- Regeneron
B cell lymphoma vaccine -- Biomira
B7-1 gene therapy –
BABS proteins -- Chiron

FIG. 28F

BAM-002 -- Novelos Therapeutics
Basiliximab (anti CD25 MAb) -- Novartis
Bay-16-9996 -- Bayer
Bay-39-9437 -- Bayer
Bay-50-4798 -- Bayer
BB-10153 -- British Biotech
BBT-001 -- Bolder BioTech.
BBT-002 -- Bolder BioTech.
BBT-003 -- Bolder BioTech.
BBT-004 -- Bolder BioTech.
BBT-005 -- Bolder BioTech.
BBT-006 -- Bolder BioTech.
BBT-007 -- Bolder BioTech.
BCH-2763 -- Shire
BCSF -- Millenium Biologix
BDNF -- Regeneron – Amgen
Becaplermin -- Johnson & Johnson, Chiron
Bectumomab – Immunomedics
Beriplast -- Aventis
Beta-adrenergic receptor gene therapy -- University of Arkansas
bFGF -- Scios
BI 51013 -- Behringwerke AG
BIBH 1 -- Boehringer Ingelheim
BIM-23190 -- Beaufour-Ipsen
birch pollen immunotherapy -- Pharmacia
bispecific fusion proteins -- NIH
Bispecific MAb 2B1 -- Chiron
Bitistatin
BIWA 4 -- Boehringer Ingelheim
blood substitute – Northfield, Baxter Intl.
BLP-25 -- Biomira
BLS-0597 -- Boston Life Sciences
BLyS -- Human Genome Sciences
BLyS radiolabelled -- Human Genome Sciences
BM 06021 -- Boehringer Mannheim
BM-202 -- BioMarin
BM-301 -- BioMarin
BM-301 -- BioMarin
BM-302 -- BioMarin
BMP 2 -- Genetics Institute/Medtronic-Sofamor Danek, Genetics Institute/ Collagenesis, Genetics Institute/Yamanouch
BMP 2 gene therapy
BMP 52 -- Aventis Pasteur, Biopharm
BMP-2 -- Genetics Institute
BMS 182248 -- Bristol-Myers Squibb
BMS 202448 -- Bristol-Myers Squibb
bone growth factors -- IsoTis
BPC-15 -- Pfizer
brain natriuretic peptide –
Breast cancer -- Oxford GlycoSciences/Medarex
Breast cancer vaccine -- Therion Biologics, Oregon
BSSL -- PPL Therapeutics
BST-2001 – BioStratum
BST-3002 -- BioStratum
BTI 322 –
butyrylcholinesterase -- Shire
C 6822 -- COR Therapeutics
C1 esterase inhibitor -- Pharming
C3d adjuvant -- AdProTech
CAB-2.1 -- Millennium
calcitonin – Inhale Therapeutics Systems, Aventis, Genetronics, TranXenoGen, Unigene, Rhone Poulenc Rohrer
calcitonin -- oral – Nobex, Emisphere, Pharmaceutical Discovery
Calcitonin gene-related peptide -- Asahi Kasei -- Unigene
calcitonin, human -- Suntory
calcitonin, nasal – Novartis, Unigene
calcitonin, Panoderm -- Elan
calcitonin, Peptitrol -- Shire
calcitonin, salmon -- Therapicon
calin -- Biopharm
Calphobindin I
calphobindin I -- Kowa
calreticulin -- NYU

FIG. 28G

Campath-1G
Campath-1M
cancer therapy -- Cangene
cancer vaccine – Aixlie, Aventis Pasteur, Center of Molecular Immunology ,YM BioSciences, Cytos, Genzyme, Transgenics, GlobeImmune, Igeneon, ImClone, Virogenetics, InterCell, Iomai, Jenner Biotherapies, Memorial Sloan-Kettering Cancer Center, Sydney Kimmel Cancer Center, Novavax, Protein Sciences, Argonex, SIGA
Cancer vaccine ALVAC-CEA B7.1 -- Aventis Pasteur/Therion Biologics
Cancer vaccine CEA-TRICOM -- Aventis Pasteur/Therion Biologics
Cancer vaccine gene therapy -- Cantab Pharmaceuticals
Cancer vaccine HER-2/neu -- Corixa
Cancer vaccine THERATOPE -- Biomira
cancer vaccine, PolyMASC -- Valentis
Candida vaccine – Corixa, Inhibitex
Canstatin -- ILEX
CAP-18 -- Panorama
Cardiovascular gene therapy -- Collateral Therapeutics
carperitide -- Suntory
Casocidin-1 -- Pharis
CAT 152 -- Cambridge Antibody Tech.
CAT 192 -- Cambridge Antibody Tech.
CAT 213 -- Cambridge Antibody Tech.
Catalase-- Enzon
Cat-PAD -- Circassia
CB 0006 -- Celltech
CCK(27-32)-- Akzo Nobel
CCR2-64I -- NIH
CD, Procept -- Paligent
CD154 gene therapy
CD39 -- Immunex
CD39-L2 -- Hyseq
CD39-L4 -- Hyseq
CD4 fusion toxin -- Senetek
CD4 IgG -- Genentech
CD4 receptor antagonists -- Pharmacopeia/Progenics
CD4 soluble -- Progenics
CD4, soluble -- Genzyme Transgenics
CD40 ligand -- Immunex
CD4-ricin chain A -- Genentech
CD59 gene therapy -- Alexion
CD8 TIL cell therapy -- Aventis Pasteur
CD8, soluble -- Avidex
CD95 ligand -- Roche
CDP 571 -- Celltech
CDP 850 -- Celltech
CDP-860 (PEG-PDGF MAb) -- Celltech
CDP 870 -- Celltech
CDS-1 -- Ernest Orlando
Cedelizumab -- Ortho-McNeil
Cetermin -- Insmed
CETP vaccine -- Avant
Cetrorelix
Cetuximab
CGH 400 -- Novartis
CGP 42934 -- Novartis
CGP 51901 – Tanox
CGRP -- Unigene
CGS 27913 -- Novartis
CGS 32359 -- Novartis
Chagas disease vaccine -- Corixa
chemokines -- Immune Response
CHH 380 -- Novartis
chitinase – Genzyme, ICOS
Chlamydia pneumoniae vaccine -- Antex Biologics
Chlamydia trachomatis vaccine -- Antex Biologics
Chlamydia vaccine -- GlaxoSmithKline
Cholera vaccine CVD 103-HgR -- Swiss Serum and Vaccine Institute Berne
Cholera vaccine CVD 112 -- Swiss Serum and Vaccine Institute Berne

FIG. 28H

Cholera vaccine inactivated oral -- SBL Vaccin
Chrysalin -- Chrysalis BioTech.
CI-782 -- Hitachi Kase
Ciliary neurotrophic factor – Fidia, Roche
CIM project -- Active Biotech
CL 329753 -- Wyeth-Ayerst
CL22, Cobra -- ML Laboratories
Clenoliximab -- IDEC
Clostridium difficile antibodies -- Epicyte
clotting factors -- Octagene
CMB 401 -- Celltech
CNTF -- Sigma-Tau
Cocaine abuse vaccine – Cantab, ImmuLogic, Scripps
coccidiomycosis vaccine -- Arizo
collagen -- Type I -- Pharming
Collagen formation inhibitors -- FibroGen
Collagen/hydroxyapatite/bone growth factor -- Aventis Pasteur, Biopharm, Orquest
collagenase -- BioSpecifics
Colorectal cancer vaccine -- Wistar Institute
Component B, Recombinant -- Serono
Connective tissue growth factor inhibitors -- FibroGen/Taisho
Contortrostatin
contraceptive vaccine -- Zonagen
Contraceptive vaccine hCG
Contraceptive vaccine male reversible -- IMMUCON
Contraceptive vaccine zona pellucida -- Zonagen
Copper-64 labelled MAb TETA-1A3 -- NCI
Coralyne
Corsevin M
C-peptide analogues -- Schwarz
CPI-1500 -- Consensus
CRF -- Neurobiological Tech.
cRGDfV pentapeptide –
CRL 1095 -- CytRx
CRL 1336 -- CytRx
CRL 1605 -- CytRx
CS-560 -- Sankyo
CSF -- ZymoGenetics
CSF-G – Hangzhou, Dong-A, Hanmi
CSF-GM – Cangene, Hunan, LG Chem
CSF-M -- Zarix
CT 1579 – Merck Frosst
CT 1786 – Merck Frosst
CT-112^ -- BTG
CTB-134L -- Xenova
CTC-111 -- Kaketsuken
CTGF -- FibroGen
CTLA4-Ig -- Bristol-Myers Squibb
CTLA4-Ig gene therapy –
CTP-37 -- AVI BioPharma
C-type natriuretic peptide -- Suntory
CVS 995 – Corvas Intl.
CX 397 – Nikko Kyodo
CY 1747 -- Epimmune
CY 1748 -- Epimmune
Cyanovirin-N
Cystic fibrosis therapy -- CBR/IVAX
CYT 351
cytokine Traps -- Regeneron
cytokines – Enzon, Cytoclonal
Cytomegalovirus glycoprotein vaccine – Chiron, Aquila Biopharmaceuticals, Aventis Pasteur, Virogenetics
Cytomegalovirus vaccine live -- Aventis Pasteur
Cytosine deaminase gene therapy -- GlaxoSmithKline
DA-3003 -- Dong-A
DAB389interleukin-6 -- Senetek
DAB389interleukin-7
DAC:GLP-2 -- ConjuChem, Inc.
Daclizumab (anti-IL2R MAb) – Protein Design Labs
DAMP^ -- Incyte Genomics
Daniplestim -- Pharmacia
darbepoetin alfa -- Amgen

FIG. 28I

DBI-3019 -- Diabetogen
DCC -- Genzyme
DDF -- Hyseq
decorin – Integra, Telios
defensins -- Large Scale Biology
DEGR-VIIa
DeImmunised antibody 3B6/22 AGEN
Deimmunised anti-cancer antibodies -- Biovation/Viragen
Dendroamide A
Dengue vaccine -- Bavarian Nordic, Merck
denileukin diftitox -- Ligand
DES-1101 -- Desmos
desirudin -- Novartis
desmopressin -- Unigene
Desmoteplase – Merck, Schering AG
Destabilase
Diabetes gene therapy – DeveloGen, Pfizer
Diabetes therapy -- Crucell
Diabetes type 1 vaccine -- Diamyd Therapeutics
DiaCIM -- YM BioSciences
dialytic oligopeptides -- Research Corp
Diamyd -- Diamyd Therapeutics
DiaPep227-- Pepgen
DiavaX -- Corixa
Digoxin MAb -- Glaxo
Diphtheria tetanus pertussis-hepatitis B vaccine -- GlaxoSmithKline
DIR therapy -- Solis Therapeutics –
DNase -- Genentech
Dornase alfa -- Genentech
Dornase alfa, inhalation -- Genentech
Doxorubicin-anti-CEA MAb conjugate – Immunomedics
DP-107 -- Trimeris
drotrecogin alfa -- Eli Lilly
DTctGMCSF
DTP-polio vaccine -- Aventis Pasteur
DU 257-KM231 antibody conjugate -- Kyowa
dural graft matrix -- Integra
Duteplase – Baxter Intl.
DWP-401 -- Daewoong
DWP-404 -- Daewoong
DWP-408 -- Daewoong
Dx 88 (Epi-KAL2) -- Dyax
Dx 890 (elastin inhibitors) -- Dyax
E coli O157 vaccine -- NIH
E21-R -- BresaGen
Eastern equine encephalitis virus vaccine –
Echicetin –
Echinhibin 1 –
Echistatin -- Merck
Echitamine –
Ecromeximab – Kyowa Hakko
EC-SOD -- PPL Therapeutics
Eculizumab (5G1.1) -- Alexion
EDF -- Ajinomoto
EDN derivative -- NIH
EDNA -- NIH
Edobacomab -- XOMA
Edrecolomab -- Centocor
EF 5077
Efalizumab -- Genentech
EGF fusion toxin – Seragen, Ligand
EGF-P64k vaccine -- Center of Molecular Immunology
EL 246 -- LigoCyte
elastase inhibitor -- Synergen
elcatonin -- Therapicon
EMD 72000 -- Merck KGaA
Emdogain -- BIORA
emfilermin -- AMRAD
Emoctakin -- Novartis
enamel matrix protein -- BIORA
Endo III -- NYU
endostatin – EntreMed, Pharis
Enhancins -- Micrologix
Enlimomab -- Isis Pharm.
Enoxaparin sodium -- Pharmuka

FIG. 28J

enzyme linked antibody nutrient depletion therapy -- KS Biomedix Holdings
Eosinophil-derived neutralizing agent –
EP-51216 -- Asta Medica
EP-51389 -- Asta Medica
EPH family ligands -- Regeneron
Epidermal growth factor -- Hitachi Kasei, Johnson & Johnson
Epidermal growth factor fusion toxin -- Senetek
Epidermal growth factor-genistein –
EPI-HNE-4 -- Dyax
EPI-KAL2 -- Dyax
Epoetin-alfa – Amgen, Dragon Pharmaceuticals, Nanjing Huaxin
Epratuzumab – Immunomedics
Epstein-Barr virus vaccine -- Aviron/SmithKline Beecham, Bioresearch
Eptacog alfa -- Novo Nordisk
Eptifibatide -- COR Therapeutics
erb-38 –
Erlizumab -- Genentech
erythropoietin -- Alkermes, ProLease, Dong-A, Elanex, Genetics Institute, LG Chem, Protein Sciences, Serono, Snow Brand, SRC VB VECTOR, Transkaryotic Therapies
Erythropoietin Beta -- Hoffman La Roche
Erythropoietin/Epoetin alfa -- Chugai
Escherichia coli vaccine -- North American Vaccine, SBL Vaccin, Swiss Serum and Vaccine Institute Berne
etanercept -- Immunex
examorelin – Mediolanum
Exendin 4 -- Amylin
exonuclease VII
F 105 -- Centocor
F-992 -- Fornix
Factor IX -- Alpha Therapeutics, Welfide Corp., CSL, enetics Institute/AHP, Pharmacia, PPL Therapeutics
Factor IX gene therapy -- Cell Genesys
Factor VII -- Novo Nordisk, Bayer, Baxter Intl.
Factor VIIa -- PPL Therapeutics, ZymoGenetics
Factor VIII – Bayer Genentech, Beaufour-Ipsen, CLB, Inex, Octagen, Pharmacia, Pharming
Factor VIII -- PEGylated -- Bayer
Factor VIII fragments -- Pharmacia
Factor VIII gene therapy -- Targeted Genetics
Factor VIII sucrose formulation – Bayer, Genentech
Factor VIII-2 -- Bayer
Factor VIII-3 -- Bayer
Factor Xa inhibitors – Merck, Novo Nordisk, Mochida
Factor XIII -- ZymoGenetics
Factors VIII and IX gene therapy -- Genetics Institute/Targeted Genetics
Famoxin -- Genset
Fas (delta) TM protein – LXR BioTech.
Fas TR -- Human Genome Sciences
Felvizumab -- Scotgen
FFR-VIIa -- Novo Nordisk
FG-001 – F-Gene
FG-002 – F-Gene
FG-004 – F-Gene
FG-005 – F-Gene
FGF + fibrin -- Repair
Fibrimage -- Bio-Tech. General
fibrin-binding peptides – ISIS Innovation
fibrinogen -- PPL Therapeutics, Pharming
fibroblast growth factor – Chiron, NYU, Ramot, ZymoGenetics
fibrolase conjugate -- Schering AG
Filgrastim -- Amgen
filgrastim -- PDA modified -- Xencor
FLT-3 ligand -- Immunex
FN18 CRM9 –

FIG. 28K

follistatin -- Biotech Australia, Human Therapeutics
follitropin alfa – Alkermes, ProLease, PowderJect, Serono, Akzo Nobel
Follitropin Beta – Bayer, Organon
FP 59
FSH -- Ferring
FSH + LH -- Ferring
F-spondin -- CeNeS
fusion protein delivery system -- UAB Research Foundation
fusion toxins -- Boston Life Sciences
G 5598 -- Genentech
GA-II -- Transkaryotic Therapies
Gamma-interferon analogues -- SRC VB VECTOR
Ganirelix -- Roche
gastric lipase -- Meristem
Gavilimomab –
G-CSF – Amgen, SRC VB VECTOR
GDF-1 -- CeNeS
GDF-5 -- Biopharm
GDNF (glial derived neurotrophic factor) -- Amgen
gelsolin -- Biogen
Gemtuzumab ozogamicin -- Celltech
Gene-activated epoetin-alfa -- Aventis Pharma -- Transkaryotic Therapies
Glanzmann thrombasthenia gene therapy –
Glatiramer acetate -- Yeda
glial growth factor 2 -- CeNeS
GLP-1 – Amylin, Suntory, TheraTech, Watson
GLP-1 peptide analogues – Zealand Pharaceuticals
GLP-2 – Novo Nordisk, Ontario, Inc., Suntory Limited
glucagon -- Eli Lilly, ZymoGenetics
Glucagon-like peptide-1 7-36 amide -- Suntory
Glucogen-like peptide -- Amylin
Glucocerebrosidase -- Genzyme
glutamate decarboxylase -- Genzyme Transgenics
Glycoprotein S3 -- Kureha
GM-CSF -- Immunex
GM-CSF tumour vaccine -- PowderJect
GnRH immunotherapeutic -- Protherics
Goserelin (LhRH antagonist) -- AstraZeneca
gp75 antigen -- ImClone
gp96 -- Antigenics
GPI 0100 -- Galenica
GR 4991W93 -- GlaxoSmithKline
Granulocyte colony-stimulating factor -- Dong-A
Granulocyte colony-stimulating factor conjugate
grass allergy therapy -- Dynavax
GRF1-44 -- ICN
Growth Factor – Chiron, Atrigel, Atrix, Innogenetics, ZymoGenetics, Novo
growth factor peptides -- Biotherapeutics
growth hormone -- LG Chem
growth hormone, Recombinant human -- Serono
GT 4086 -- Gliatech
GW 353430 -- GlaxoSmithKline
GW-278884 -- GlaxoSmithKline
H 11 -- Viventia Biotech
H5N1 influenza A virus vaccine -- Protein Sciences
haemoglobin -- Biopure
haemoglobin 3011, Recombinant -- Baxter Healthcare
haemoglobin crosfumaril – Baxter Intl.
haemoglobin stabilized -- Ajinomoto
haemoglobin, recombinant -- Apex
HAF -- Immune Response
Hantavirus vaccine
HB 19
HBNF -- Regeneron
HCC-1 -- Pharis

FIG. 28L

hCG -- Milkhaus
hCG vaccine -- Zonagen
HE-317 -- Hollis-Eden Pharmaceuticals
Heat shock protein cancer and influenza vaccines -- StressGen
Helicobacter pylori vaccine -- Acambis, AstraZeneca/CSL, Chiron, Provalis
Helistat-G -- GalaGen
Hemolink -- Hemosol
hepapoietin -- Snow Brand
heparanase -- InSight
heparinase I -- Ibex
heparinase III -- Ibex
Hepatitis A vaccine -- American Biogenetic Sciences
Hepatitis A vaccine inactivated
Hepatitis A vaccine Nothav -- Chiron
Hepatitis A-hepatitis B vaccine -- GlaxoSmithKline
hepatitis B therapy -- Tripep
Hepatitis B vaccine – Amgen, Chiron SpA, Meiji Milk, NIS, Prodeva, PowderJect, Rhein Biotech
Hepatitis B vaccine recombinant -- Evans Vaccines, Epitec Combiotech, Genentech, MedImmune, Merck Sharp & Dohme, Rhein Biotech, Shantha Biotechnics, Vector, Yeda
Hepatitis B vaccine recombinant TGP 943 -- Takeda
Hepatitis C vaccine -- Bavarian Nordic, Chiron, Innogenetics Acambis,
Hepatitis D vaccine -- Chiron Vaccines
Hepatitis E vaccine recombinant -- Genelabs/GlaxoSmithKline, Novavax
hepatocyte growth factor – Panorama, Sosei
hepatocyte growth factor kringle fragments -- EntreMed
Her-2/Neu peptides -- Corixa
Herpes simplex glycoprotein DNA vaccine – Merck, Wyeth-Lederle Vaccines-Malvern, Genentech, GlaxoSmithKline, Chiron, Takeda
Herpes simplex vaccine -- Cantab Pharmaceuticals, CEL-SCI, Henderson Morley
Herpes simplex vaccine live -- ImClone Systems/Wyeth-Lederle, Aventis Pasteur
HGF derivatives -- Dompe
hIAPP vaccine -- Crucell
Hib-hepatitis B vaccine -- Aventis Pasteur
HIC 1
HIP-- Altachem
Hirudins – Biopharma, Cangene, Dongkook, Japan Energy Corporation, Pharmacia Corporation, SIR International, Sanofi-Synthelabo, Sotragene, Rhein Biotech
HIV edible vaccine -- ProdiGene
HIV gp120 vaccine – Chiron, Ajinomoto, GlaxoSmithKline, ID Vaccine, Progenics, VaxGen
HIV gp120 vaccine gene therapy –
HIV gp160 DNA vaccine – PowderJect, Aventis Pasteur, Oncogen, Hyland Immuno, Protein Sciences
HIV gp41 vaccine -- Panacos
HIV HGP-30W vaccine -- CEL-SCI
HIV immune globulin – Abbott, Chiron
HIV peptides -- American Home Products
HIV vaccine -- Applied bioTech., Axis Genetics, Biogen, Bristol-Myers Squibb, Genentech, Korea Green Cross, NIS, Oncogen, Protein Sciences Corporation, Terumo, Tonen Corporation, Wyeth-Ayerst, Wyeth-Lederle Vaccines-Malvern, Advanced BioScience Laboratories, Bavarian Nordic, Bavarian Nordic/Statens Serum Institute, GeneCure, Immune Response, Progenics, Therion Biologics, United Biomedical, Chiron

FIG. 28M

HIV vaccine vCP1433 -- Aventis Pasteur
HIV vaccine vCP1452 -- Aventis Pasteur
HIV vaccine vCP205 -- Aventis Pasteur
HL-9 -- American BioScience
HM-9239 -- Cytran
HML-103 -- Hemosol
HML-104 -- Hemosol
HML-105 -- Hemosol
HML-109 -- Hemosol
HML-110 -- Hemosol
HML-121 -- Hemosol
hNLP -- Pharis
Hookworm vaccine
host-vector vaccines -- Henogen
HPM 1 -- Chugai
HPV vaccine -- MediGene
HSA -- Meristem
HSF -- StressGen
HSP carriers –Weizmann, Yeda, Peptor
HSPPC-70 -- Antigenics
HSPPC-96, pathogen-derived -- Antigenics
HSV 863 -- Novartis
HTLV-I DNA vaccine
HTLV-I vaccine
HTLV-II vaccine -- Access
HU 901 -- Tanox
Hu23F2G -- ICOS
HuHMFG1
HumaLYM -- Intracell
Human krebs statika -- Yamanouchi
human monoclonal antibodies -- Abgenix/Biogen, Abgenix/ Corixa, Abgenix/Immunex, Abgenix/Lexicon, Abgenix/ Pfizer, Athersys/Medarex, Biogen/MorphoSys, CAT/Searle, Centocor/Medarex, Corixa/Kirin Brewery, Corixa/Medarex, Eos BioTech./Medarex, Eos/Xenerex, Exelixis/Protein Design Labs, ImmunoGen/ Raven, Medarex/ B.Twelve, MorphoSys/ImmunoGen, XTL Biopharmaceuticals/Dyax,
Human monoclonal antibodies -- Medarex/Northwest Biotherapeutics, Medarex/Seattle Genetics
human netrin-1 -- Exelixis
human papillomavirus antibodies -- Epicyte
Human papillomavirus vaccine -- Biotech Australia, IDEC, StressGen
Human papillomavirus vaccine MEDI 501 -- MedImmune/GlaxoSmithKline
Human papillomavirus vaccine MEDI 503/MEDI 504 -- MedImmune/GlaxoSmithKline
Human papillomavirus vaccine TA-CIN – Cantab Pharmaceuticals
Human papillomavirus vaccine TA-HPV -- Cantab Pharmaceuticals
Human papillomavirus vaccine TH-GW -- Cantab/GlaxoSmithKline
human polyclonal antibodies -- Biosite/Eos BioTech./ Medarex
human type II anti factor VIII monoclonal antibodies -- ThromboGenics
humanised anti glycoprotein Ib murine monoclonal antibodies -- ThromboGenics
HumaRAD -- Intracell
HuMax EGFR -- Genmab
HuMax-CD4 -- Medarex
HuMax-IL15 -- Genmab
HYB 190 -- Hybridon
HYB 676 -- Hybridon
I-125 MAb A33 -- Celltech
Ibritumomab tiuxetan -- IDEC
IBT-9401 -- Ibex
IBT-9402 -- Ibex
IC 14 -- ICOS
Idarubicin anti-Ly-2.1 –
IDEC 114 -- IDEC
IDEC 131 -- IDEC
IDEC 152 -- IDEC
IDM 1 -- IDM
IDPS -- Hollis-Eden Pharmaceuticals

FIG. 28N

iduronate-2-sulfatase -- Transkaryotic Therapies
IGF/IBP-2-13 -- Pharis
IGN-101 -- Igeneon
IK HIR02 -- Iketon
IL-11 -- Genetics Institute/AHP
IL-13-PE38 -- NeoPharm
IL-17 receptor -- Immunex
IL-18BP -- Yeda
IL-1Hy1 -- Hyseq
IL-1ß -- Celltech
IL-1ß adjuvant -- Celltech
IL-2 -- Chiron
IL-2 + IL-12 -- Hoffman La-Roche
IL-6/sIL-6R fusion -- Hadasit
IL-6R derivative -- Tosoh
IL-7-Dap 389 fusion toxin – Ligand
IL-21 – Novo Nordisk, ZymoGenetics
IM-862 -- Cytran
IMC-1C11 -- ImClone
imiglucerase -- Genzyme
Immune globulin intravenous (human) -- Hoffman La Roche
immune privilege factor -- Proneuron
Immunocal -- Immunotec
Immunogene therapy -- Briana Bio-Tech
Immunoliposomal 5-fluorodeoxyuridine-dipalmitate –
immunosuppressant vaccine -- Aixlie
immunotoxin – Antisoma, NIH
ImmuRAIT-Re-188 – Immunomedics
imreg-1 -- Imreg
infertility -- Johnson & Johnson, E-TRANS
Infliximab -- Centocor
Influenza virus vaccine -- Aventis Pasteur, Protein Sciences
inhibin -- Biotech Australia, Human Therapeutics
Inhibitory G protein gene therapy
INKP-2001 -- InKine
Inolimomab -- Diaclone
insulin -- AutoImmune, Altea, Biobras, BioSante, Bio-Tech. General, Chong Kun Dang, Emisphere, Flamel, Provalis, Rhein Biotech, TranXenoGen
insulin (bovine) -- Novartis
insulin analogue -- Eli Lilly
Insulin Aspart -- Novo Nordisk
insulin detemir -- Novo Nordisk
insulin glargine -- Aventis
insulin inhaled – Inhale Therapeutics Systems, Alkermes
insulin oral -- Inovax
insulin, AeroDose -- AeroGen
insulin, AERx -- Aradigm
insulin, BEODAS -- Elan
insulin, Biphasix -- Helix
insulin, buccal -- Generex
insulin, I2R -- Flemington
insulin, intranasal -- Bentley
insulin, oral – Nobex, Unigene
insulin, Orasome -- Endorex
insulin, ProMaxx -- Epic
insulin, Quadrant -- Elan
insulin, recombinant -- Aventis
insulin, Spiros -- Elan
insulin, Transfersome -- IDEA
insulin, Zymo, recombinant -- Novo Nordisk
insulinotropin -- Scios
Insulysin gene therapy –
integrin antagonists -- Merck
interferon (Alpha2) -- SRC VB VECTOR, Viragen, Dong-A, Hoffman La-Roche, Genentech
interferon – BioMedicines, Human Genome Sciences
interferon (Alfa-n3)—Interferon Sciences Intl.
interferon (Alpha), Biphasix -- Helix

FIG. 28O

interferon (Alpha)—Amgen, BioNative, Novartis, Genzyme Transgenics, Hayashibara, Inhale Therapeutics Systems, Medusa, Flamel, Dong-A, GeneTrol, Nastech, Shantha, Wassermann, LG Chem, Sumitomo, Aventis, Behring EGIS, Pepgen, Servier, Rhein Biotech,
interferon (Alpha2A)
interferon (Alpha2B) – Enzon, Schering-Plough, Biogen, IDEA
interferon (Alpha-N1) -- GlaxoSmithKline
interferon (beta) – Rentschler, GeneTrol, Meristem, Rhein Biotech, Toray, Yeda, Daiichi, Mochida
interferon (Beta1A) – Serono, Biogen
interferon (beta1A),inhale -- Biogen
interferon (ß1b)-- Chiron
interferon (tau)-- Pepgen
Interferon alfacon-1 -- Amgen
Interferon alpha-2a vaccine
Interferon Beta 1b -- Schering/Chiron, InterMune
Interferon Gamma -- Boehringer Ingelheim, Sheffield, Rentschler, Hayashibara
interferon receptor , Type I -- Serono
interferon(Gamma1B) -- Genentech
Interferon-alpha-2b + ribavirin – Biogen, ICN
Interferon-alpha-2b gene therapy -- Schering-Plough
Interferon-con1 gene therapy –
interleukin-1 antagonists -- Dompe
Interleukin-1 receptor antagonist -- Abbott Bioresearch, Pharmacia
Interleukin-1 receptor type I -- Immunex
interleukin-1 receptor Type II -- Immunex
Interleukin-1 trap -- Regeneron
Interleukin-1-alpha -- Immunex/Roche
interleukin-2 -- SRC VB VECTOR, Ajinomoto, Biomira, Chiron
IL-2/ diphtheria toxin -- Ligand
Interleukin-3 -- Cangene
Interleukin-4 -- Immunology Ventures, Sanofi Winthrop, Schering-Plough, Immunex/ Sanofi Winthrop, Bayer, Ono
interleukin-4 + TNF-Alpha -- NIH
interleukin-4 agonist -- Bayer
interleukin-4 fusion toxin -- Ligand
Interleukin-4 receptor – Immunex, Immun
Interleukin-6 – Ajinomoto, Cangene, Yeda, Genetics Institute, Novartis
interleukin-6 fusion protein
interleukin-6 fusion toxin – Ligand, Serono
interleukin-7 -- IC Innovations
interleukin-7 receptor -- Immunex
interleukin-8 antagonists -- Kyowa Hakko/Millennium/Pfizer
interleukin-9 antagonists -- Genaera
Interleukin-10 – DNAX, Schering-Plough
Interleukin-10 gene therapy –
interleukin-12 -- Genetics Institute, Hoffman La-Roche
interleukin-13 -- Sanofi
interleukin-13 antagonists -- AMRAD
Interleukin-13-PE38QQR
interleukin-15 -- Immunex
interleukin-16 -- Research Corp
interleukin-18 -- GlaxoSmithKline
Interleukin-18 binding protein -- Serono
Ior-P3 -- Center of Molecular Immunology
IP-10 -- NIH
IPF -- Metabolex
IR-501 -- Immune Response
ISIS 9125 -- Isis Pharmaceuticals
ISURF No. 1554 -- Millennium
ISURF No. 1866 – Iowa State Univer.
ITF-1697 -- Italfarmaco
IxC 162 -- Ixion
J 695 -- Cambridge Antibody Tech., Genetics Inst., Knoll
Jagged + FGF -- Repair

FIG. 28P

JKC-362 -- Phoenix Pharmaceuticals
JTP-2942 – Japan Tobacce
Juman monoclonal antibodies -- Medarex/Raven
K02 -- Axys Pharmaceuticals
Keliximab -- IDEC
Keyhole limpet haemocyanin
KGF -- Amgen
KM 871 -- Kyowa
KPI 135 -- Scios
KPI-022 -- Scios
Kringle 5
KSB 304
KSB-201 -- KS Biomedix
L 696418 -- Merck
L 703801 -- Merck
L1 -- Acorda
L-761191 -- Merck
lactoferrin – Meristem, Pharming, Agennix
lactoferrin cardio -- Pharming
LAG-3 -- Serono
LAIT -- GEMMA
LAK cell cytotoxin -- Arizona
lamellarins -- PharmaMar/University of Malaga
laminin A peptides -- NIH
lanoteplase -- Genetics Institute
laronidase -- BioMarin
Lassa fever vaccine
LCAT -- NIH
LDP 01 -- Millennium
LDP 02 -- Millennium
Lecithinized superoxide dismutase -- Seikagaku
LeIF adjuvant -- Corixa
leishmaniasis vaccine -- Corixa
lenercept -- Hoffman La-Roche
Lenograstim – Aventis, Chugai
lepirudin -- Aventis
leptin – Amgen, IC Innovations
Leptin gene therapy -- Chiron Corporation
leptin, 2nd-generation -- Amgen
leridistim -- Pharmacia
leuprolide, ProMaxx -- Epic
leuprorelin, oral -- Unigene
LeuTech -- Papatin
LEX 032 -- SuperGen
LiDEPT -- Novartis
Lintuzumab (anti-CD33 MAb) -- Protein Design Labs
lipase -- Altus Biologics
lipid A vaccine -- EntreMed
lipid-linked anchor Tech. – ICRT, ID Biomedical
liposome-CD4 Tech. -- Sheffield
Listeria monocytogenes vaccine
LMB 1
LMB 7
LMB 9 -- Battelle Memorial Institute, NIH
LM-CD45 -- Cantab Pharmaceuticals
lovastatin -- Merck
LSA-3
LT-ß receptor -- Biogen
lung cancer vaccine -- Corixa
lusupultide -- Scios
L-Vax -- AVAX
LY 355455 -- Eli Lilly
LY 366405 -- Eli Lilly
LY-355101 -- Eli Lilly
Lyme disease DNA vaccine -- Vical/Aventis Pasteur
Lyme disease vaccine -- Aquila Biopharmaceuticals, Aventis, Pasteur, Symbicom, GlaxoSmithKline, Hyland Immuno, MedImmune
Lymphocytic choriomeningitis virus vaccine
lymphoma vaccine – Biomira, Genitope
LYP18
lys plasminogen, recombinant
Lysosomal storage disease gene therapy -- Avigen
lysostaphin -- Nutrition 21

FIG. 28Q

M 23 -- Gruenenthal
M1 monoclonal antibodies -- Acorda Therapeutics
MA 16N7C2 – Corvas Intl.
malaria vaccine -- GlaxoSmithKline, AdProTech, Antigenics, Apovia, Aventis Pasteur, Axis Genetics, Behringwerke, CDCP, Chiron Vaccines, Genzyme Transgenics, Hawaii, MedImmune, NIH, NYU, Oxxon, Roche/Saramane, Biotech Australia, Rx Tech
Malaria vaccine CDC/NIIMALVAC-1
malaria vaccine,multicomponent
mammaglobin -- Corixa
mammastatin -- Biotherapeutics
mannan-binding lectin -- NatImmu
mannan-MUC1 -- Psiron
MAP 30
Marinovir -- Phytera
MARstem -- Maret
MB-015 -- Mochida
MBP -- ImmuLogic
MCI-028 -- Mitsubishi-Tokyo
MCIF -- Human Genome Sciences
MDC -- Advanced BioScience -- Akzo Nobel, ICOS
MDX 11 -- Medarex
MDX 210 -- Medarex
MDX 22 -- Medarex
MDX 22
MDX 240 -- Medarex
MDX 33
MDX 44 -- Medarex
MDX 447 -- Medarex
MDX H210 -- Medarex
MDX RA -- Houston BioTech., Medarex
ME-104 -- Pharmexa
Measles vaccine
Mecasermin -- Cephalon/Chiron, Chiron
MEDI 488 -- MedImmune
MEDI 500
MEDI 507 -- BioTransplant
melanin concentrating hormone -- Neurocrine Biosciences
melanocortins -- OMRF
Melanoma monoclonal antibodies -- Viragen
melanoma vaccine -- GlaxoSmithKline, Akzo Nobel, Avant, Aventis Pasteur, Bavarian Nordic, Biovector, CancerVax, Genzyme Molecular Oncology, Humbolt, ImClone Systems, Memorial, NYU, Oxxon
Melanoma vaccine Magevac -- Therion
memory enhancers -- Scios
meningococcal B vaccine -- Chiron
meningococcal vaccine -- CAMR
Meningococcal vaccine group B conjugate -- North American Vaccine
Meningococcal vaccine group B recombinant -- BioChem Vaccines, Microscience
Meningococcal vaccine group Y conjugate -- North American Vaccine
Meningococcal vaccine groups A B and C conjugate -- North American Vaccine
Mepolizumab -- GlaxoSmithKline
Metastatin – EntreMed, Takeda
Met-CkB7 -- Human Genome Sciences
met-enkephalin -- TNI
METH-1 -- Human Genome Sciences
methioninase -- AntiCancer
Methionine lyase gene therapy -- AntiCancer
Met-RANTES – Genexa Biomedical, Serono
Metreleptin
Microtubule inhibitor MAb Immunogen/Abgenix
MGDF -- Kirin
MGV -- Progenics
micrin -- Endocrine
microplasmin -- ThromboGenics
MIF -- Genetics Institute

FIG. 28R

migration inhibitory factor -- NIH
Mim CD4.1 – Xycte Therapies
mirostipen -- Human Genome Sciences
Mitumomab (BEC-2) – ImClone Systems, Merck KGaA
MK 852 -- Merck
MLN 1202 (Anti-CCR2 monoclonal antibody) – Millenium Pharmaceuticals
Mobenakin -- NIS
molgramostim -- Genetics Institute, Novartis
monoclonal antibodies -- Abgenix/Celltech, Immusol/ Medarex, Viragen/ Roslin Institute, Cambridge Antibody Tech./Elan
MAb 108 –
MAb 10D5 --
MAb 14.18-interleukin-2 immunocytokine -- Lexigen
MAb 14G2a –
MAb 15A10 –
MAb 170 -- Biomira
MAb 177Lu CC49 --
MAb 17F9
MAb 1D7
MAb 1F7 – Immune Network
MAb 1H10-doxorubicin conjugate
MAb 26-2F
MAb 2A11
MAb 2E1 -- RW Johnson
MAb 2F5
MAb 31.1 -- International BioImmune Systems
MAb 32 -- Cambridge Antibody Tech., Peptech
MAb 323A3 -- Centocor
MAb 3C5
MAb 3F12
MAb 3F8
MAb 42/6
MAb 425 -- Merck KGaA
MAb 447-52D -- Merck Sharp & Dohme
MAb 45-2D9- – haematoporphyrin conjugate
MAb 4B4
MAb 4E3-CPA conjugate -- BCM Oncologia
MAb 4E3-daunorubicin conjugate
MAb 50-6
MAb 50-61A – Institut Pasteur
MAb 5A8 -- Biogen
MAb 791T/36-methotrexate conjugate
MAb 7c11.e8
MAb 7E11 C5-selenocystamine conjugate
MAb 93KA9 -- Novartis
MAb A5B7-cisplatin conjugate -- Biodynamics Research, Pharmacia
MAb A5B7-I-131
MAb A7
MAb A717 -- Exocell
MAb A7-zinostatin conjugate
MAb ABX-RB2 -- Abgenix
MAb ACA 11
MAb AFP-I-131 – Immunomedics
MAb AP1
MAb AZ1
MAb B3-LysPE40 conjugate
MAb B4 – United Biomedical
MAb B43 Genistein-conjugate
MAb B43.13-Tc-99m -- Biomira
MAb B43-PAP conjugate
MAb B4G7-gelonin conjugate
MAb BCM 43-daunorubicin conjugate -- BCM Oncologia
MAb BIS-1
MAb BMS 181170 -- Bristol-Myers Squibb
MAb BR55-2
MAb BW494
MAb C 242-DM1 conjugate -- ImmunoGen
MAb C242-PE conjugate
MAb c30-6
MAb CA208-cytorhodin-S conjugate -- Hoechst Japan
MAb CC49 -- Enzon

FIG. 28S

MAb ch14.18 –
MAb CH14.18-GM-CSF fusion protein -- Lexigen
MAb chCE7
MAb CI-137 -- AMRAD
MAb cisplatin conjugate
MAb CLB-CD19
MAb CLB-CD19v
MAb CLL-1 -- Peregrine
MAb CLL-1-GM-CSF conjugate
MAb CLL-1-IL-2 conjugate -- Peregrine
MAb CLN IgG -- doxorubicin conjugates
MAb conjugates – Tanox
MAb D612
MAb Dal B02
MAb DC101 -- ImClone
MAb EA 1 –
MAb EC708 -- Biovation
MAb EP-5C7 -- Protein Design Labs
MAb ERIC-1 -- ICRT
MAb F105 gene therapy
MAb FC 2.15
MAb G250 -- Centocor
MAb GA6
MAb GA733
MAb Gliomab-H -- Viventia Biotech
MAb HB2-saporin conjugate
MAb HD 37 –
MAb HD37-ricin chain-A conjugate
MAb HNK20 -- Acambis
MAb huN901-DM1 conjugate -- ImmunoGen
MAb I-131 CC49 -- Corixa
MAb ICO25
MAb ICR12-CPG2 conjugate
MAb ICR-62
MAb IRac-ricin A conjugate
MAb K1
MAb KS1-4-methotrexate conjugate
MAb L6 -- Bristol-Myers Squibb, Oncogen
MAb LiCO 16-88
MAb LL2-I-131 – Immunomedics
MAb LL2-Y-90
MAb LS2D617 -- Hybritech
MAb LYM-1-gelonin conjugate
MAb LYM-1-I-131
MAb LYM-1-Y-90
MAb LYM-2 -- Peregrine
MAb M195
MAb M195-bismuth 213 conjugate -- Protein Design Labs
MAb M195-gelonin conjugate
MAb M195-I-131
MAb M195-Y-90
MAb MA 33H1 -- Sanofi
MAb MAD11
MAb MGb2
MAb MINT5
MAb MK2-23
MAb MOC31 ETA(252-613) conjugate
MAb MOC-31-In-111
MAb MOC-31-PE conjugate
MAb MR6 –
MAb MRK-16 -- Aventis Pasteur
MAb MS11G6
MAb MX-DTPA BrE-3
MAb MY9
MAb Nd2 -- Tosoh
MAb NG-1 -- Hygeia
MAb NM01 – Nissin Food
MAb OC 125
MAb OC 125-CMA conjugate
MAb OKI-1 -- Ortho-McNeil
MAb OX52 -- Bioproducts for Science
MAb PMA5
MAb PR1
MAb prost 30
MAb R-24
MAb R-24 α Human GD3 -- Celltech
MAb RFB4-ricin chain A conjugate
MAb RFT5-ricin chain A conjugate
MAb SC 1

FIG. 28T

MAb SM-3 -- ICRT
MAb SMART 1D10 -- Protein Design Labs
MAb SMART ABL 364 -- Novartis
MAb SN6f
MAb SN6f-deglycosylated ricin A chain conjugate –
MAb SN6j
MAb SN7-ricin chain A conjugate
MAb T101-Y-90 conjugate -- Hybritech
MAb T-88 -- Chiron
MAb TB94 -- Cancer ImmunoBiology
MAb TEC 11
MAb TES-23 -- Chugai
MAb TM31 -- Avant
MAb TNT-1 -- Cambridge Antibody Tech., Peregrine
MAb TNT-3
MAb TNT-3 -- IL2 fusion protein –
MAb TP3-At-211
MAb TP3-PAP conjugate –
MAb UJ13A -- ICRT
MAb UN3
MAb ZME-018-gelonin conjugate
MAb-BC2 -- GlaxoSmithKline
MAb-DM1 conjugate -- ImmunoGen
MAb-ricin-chain-A conjugate -- XOMA
MAb-temoporfin conjugates
Monopharm C -- Viventia Biotech
monteplase -- Eisai
montirelin hydrate -- Gruenenthal
moroctocog alfa -- Genetics Institute
Moroctocog-alfa -- Pharmacia
MP 4
MP-121 -- Biopharm
MP-52 -- Biopharm
MRA -- Chugai
MS 28168 -- Mitsui Chemicals, Nihon Schering
MSH fusion toxin -- Ligand
MSI-99 -- Genaera
MT 201 -- Micromet
Muc-1 vaccine -- Corixa
mucosal tolerance -- Aberdeen
mullerian inhibiting subst
muplestim -- Genetics Institute, Novartis, DSM Anti-Infectives
murine MAb -- KS Biomedix
Mutant somatropin -- JCR Pharmaceutical
MV 833 -- Toagosei
Mycoplasma pulmonis vaccine
Mycoprex -- XOMA
myeloperoxidase -- Henogen
myostatin -- Genetics Institute
Nacolomab tafenatox -- Pharmacia
Nagrecor -- Scios
nagrestipen -- British Biotech
NAP-5 – Corvas Intl.
NAPc2 – Corvas Intl.
nartograstim -- Kyowa
Natalizumab -- Protein Design Labs
Nateplase – NIH, Nihon Schering
nateplase -- Schering AG
NBI-3001 -- Neurocrine Biosci.
NBI-5788 -- Neurocrine Biosci.
NBI-6024 -- Neurocrine Biosci.
Nef inhibitors -- BRI
Neisseria gonorrhoea vaccine -- Antex Biologics
Neomycin B-arginine conjugate
Nerelimomab -- Chiron
Nerve growth factor – Amgen – Chiron, Genentech
Nerve growth factor gene therapy
nesiritide citrate -- Scios
neuregulin-2 -- CeNeS
neurocan -- NYU
neuronal delivery system -- CAMR
Neurophil inhibitory Factor -- Corvas
Neuroprotective vaccine -- University of Auckland
neurotrophic chimaeras -- Regeneron
neurotrophic factor – NsGene, CereMedix

FIG. 28U

NeuroVax -- Immune Response
neurturin -- Genentech
neutral endopeptidase -- Genentech
NGF enhancers -- NeuroSearch
NHL vaccine -- Large Scale Biology
NIP45 -- Boston Life Sciences
NKI-B20
NM 01 – Nissin Food
NMI-139 -- NitroMed
NMMP -- Genetics Institute
NN-2211 -- Novo Nordisk
Noggin -- Regeneron
Nonacog alfa
Norelin -- Biostar
Norwalk virus vaccine
NRLU 10 -- NeoRx
NRLU 10 PE -- NeoRx
NT-3 -- Regeneron
NT-4/5 -- Genentech
NU 3056
NU 3076
NX 1838 -- Gilead Sciences
NY ESO-1/CAG-3 antigen -- NIH
NYVAC-7 -- Aventis Pasteur
NZ-1002 -- Novazyme
obesity therapy -- Nobex
OC 10426 -- Ontogen
OC 144093 -- Ontogen
OCIF -- Sankyo
Oct-43 -- Otsuka
Odulimomab -- Immunotech
OK PSA - liposomal
OKT3-gamma-1-ala-ala
OM 991
OM 992
Omalizumab -- Genentech
oncoimmunin-L -- NIH
Oncolysin B -- ImmunoGen
Oncolysin CD6 -- ImmunoGen
Oncolysin M -- ImmunoGen
Oncolysin S -- ImmunoGen
Oncophage -- Antigenics
Oncostatin M -- Bristol-Myers Squibb
OncoVax-CL -- Jenner Biotherapies
OncoVax-P -- Jenner Biotherapies
onercept -- Yeda
onychomycosis vaccine -- Boehringer Ingelheim
opebecan -- XOMA
opioids -- Arizona
Oprelvekin -- Genetics Institute
Oregovomab -- AltaRex
Org-33408 b-- Akzo Nobel
Orolip DP -- EpiCept
oryzacystatin
OSA peptides – GenSci Regeneration
osteoblast-cadherin GF -- Pharis
Osteocalcin-thymidine kinase gene therapy
osteogenic protein -- Curis
osteopontin -- OraPharma
osteoporosis peptides – Integra, Telios
osteoprotegerin – Amgen, SnowBrand
otitis media vaccines -- Antex Biologics
ovarian cancer -- University of Alabama
OX40-IgG fusion protein -- Cantab, Xenova
P 246 -- Diatide
P 30 -- Alfacell
p1025 -- Active Biotech
P-113^ -- Demegen
P-16 peptide -- Transition Therapeutics
p43 -- Ramot
P-50 peptide -- Transition Therapeutics
p53 + RAS vaccine -- NIH, NCI
PACAP(1-27) analogue
paediatric vaccines -- Chiron
Pafase -- ICOS
PAGE-4 plasmid DNA -- IDEC
PAI-2 -- Biotech Australia, Human Therapeutics
Palifermin (keratinocyte growth factor) -- Amgen
Palivizumab -- MedImmune

FIG. 28V

PAM 4 -- Merck
pamiteplase -- Yamanouchi
pancreatin, Minitabs -- Eurand
Pangen -- Fournier
Pantarin – Selective Genetics
Parainfluenza virus vaccine – Pharmacia, Pierre Fabre
paraoxanase -- Esperion
parathyroid hormone – Abiogen, Korea Green Cross
Parathyroid hormone (1-34) -- Chugai/Suntory
Parkinson's disease gene therapy -- Cell Genesys/ Ceregene
Parvovirus vaccine -- MedImmune
PCP-Scan – Immunomedics
PDGF -- Chiron
PDGF cocktail -- Theratechnologies
peanut allergy therapy -- Dynavax
PEG anti-ICAM MAb -- Boehringer Ingelheim
PEG asparaginase -- Enzon
PEG glucocerebrosidase
PEG hirudin – Knoll
PEG interferon-alpha-2a -- Roche
PEG interferon-alpha-2b + ribavirin – Biogen, Enzon, ICN Pharmaceuticals, Schering-Plough
PEG MAb A5B7 –
Pegacaristim – Amgen -- Kirin Brewery -- ZymoGenetics
Pegaldesleukin -- Research Corp
pegaspargase -- Enzon
pegfilgrastim -- Amgen
PEG-interferon Alpha -- Viragen
PEG-interferon Alpha 2A -- Hoffman La-Roche
PEG-interferon Alpha 2B -- Schering-Plough
PEG-r-hirudin -- Abbott
PEG-rHuMGDF -- Amgen
PEG-uricase -- Mountain View
Pegvisomant – Genentech
PEGylated proteins, PolyMASC -- Valentis
PEGylated recombinant native human leptin -- Roche
Pemtumomab
Penetratin -- Cyclacel
Pepscan – Antisoma
peptide G – Peptech, ICRT
peptide vaccine -- NIH ,NCI
Pexelizumab
pexiganan acetate -- Genaera
Pharmaprojects No. 3179 -- NYU
Pharmaprojects No. 3390 -- Ernest Orlando
Pharmaprojects No. 3417 -- Sumitomo
Pharmaprojects No. 3777 -- Acambis
Pharmaprojects No. 4209 -- XOMA
Pharmaprojects No. 4349 – Baxter Intl.
Pharmaprojects No. 4651
Pharmaprojects No. 4915 -- Avanir
Pharmaprojects No. 5156 -- Rhizogenics
Pharmaprojects No. 5200 -- Pfizer
Pharmaprojects No. 5215 -- Origene
Pharmaprojects No. 5216 -- Origene
Pharmaprojects No. 5218 -- Origene
Pharmaprojects No. 5267 -- ML Laboratories
Pharmaprojects No. 5373 -- MorphoSys
Pharmaprojects No. 5493 -- Metabolex
Pharmaprojects No. 5707 -- Genentech
Pharmaprojects No. 5728 -- Autogen
Pharmaprojects No. 5733 -- BioMarin
Pharmaprojects No. 5757 -- NIH
Pharmaprojects No. 5765 -- Gryphon
Pharmaprojects No. 5830 -- AntiCancer
Pharmaprojects No. 5839 -- Dyax
Pharmaprojects No. 5849 -- Johnson & Johnson
Pharmaprojects No. 5860 -- Mitsubishi-Tokyo

FIG. 28W

Pharmaprojects No. 5869 – Oxford GlycoSciences
Pharmaprojects No. 5883 -- Asahi Brewery
Pharmaprojects No. 5947 -- StressGen
Pharmaprojects No. 5961 -- Theratechnologies
Pharmaprojects No. 5962 -- NIH
Pharmaprojects No. 5966 -- NIH
Pharmaprojects No. 5994 -- Pharming
Pharmaprojects No. 5995 -- Pharming
Pharmaprojects No. 6023 -- IMMUCON
Pharmaprojects No. 6063 -- Cytoclonal
Pharmaprojects No. 6073 -- SIDDCO
Pharmaprojects No. 6115 -- Genzyme
Pharmaprojects No. 6227 -- NIH
Pharmaprojects No. 6230 -- NIH
Pharmaprojects No. 6236 -- NIH
Pharmaprojects No. 6243 -- NIH
Pharmaprojects No. 6244 -- NIH
Pharmaprojects No. 6281 -- Senetek
Pharmaprojects No. 6365 -- NIH
Pharmaprojects No. 6368 -- NIH
Pharmaprojects No. 6373 -- NIH
Pharmaprojects No. 6408 – Pan Pacific
Pharmaprojects No. 6410 -- Athersys
Pharmaprojects No. 6421 – Oxford GlycoSciences
Pharmaprojects No. 6522 -- Maxygen
Pharmaprojects No. 6523 -- Pharis
Pharmaprojects No. 6538 -- Maxygen
Pharmaprojects No. 6554 -- APALEXO
Pharmaprojects No. 6560 -- Ardana
Pharmaprojects No. 6562 -- Bayer
Pharmaprojects No. 6569 -- Eos
Phenoxazine
Phenylase -- Ibex
Pigment epithelium derived factor – plasminogen activator inhibitor-1, recombinant -- DuPont Pharmaceuticals
Plasminogen activators -- Abbott Laboratories, American Home Products, Boehringer Mannheim, Chiron Corporation, DuPont Pharmaceuticals, Eli Lilly, Shionogi, Genentech, Genetics Institute, GlaxoSmithKline, Hemispherx Biopharma, Merck & Co, Novartis, Pharmacia Corporation, Wakamoto, Yeda
plasminogen-related peptides -- Bio-Tech. General/MGH
platelet factor 4 -- RepliGen
Platelet-derived growth factor – Amgen -- ZymoGenetics
plusonermin-- Hayashibara
PMD-2850 -- Protherics
Pneumococcal vaccine -- Antex Biologics, Aventis Pasteur
Pneumococcal vaccine intranasal -- BioChem Vaccines/Biovector
PR1A3
PR-39
pralmorelin -- Kaken
Pretarget-Lymphoma -- NeoRx
Priliximab -- Centocor
PRO 140 -- Progenics
PRO 2000 -- Procept
PRO 367 -- Progenics
PRO 542 -- Progenics
pro-Apo A-I -- Esperion
prolactin -- Genzyme
Prosaptide TX14(A) -- Bio-Tech. General
prostate cancer antbodies – Immunex, UroCor
prostate cancer antibody therapy -- Genentech/UroGenesys, Genotherapeutics
prostate cancer immunotherapeutics -- The PSMA Development Company
prostate cancer vaccine -- Aventis Pasteur, Zonagen, Corixa, Dendreon, Jenner Biotherapies, Therion Biologics

FIG. 28X

prostate-specific antigen -- EntreMed
protein A -- RepliGen
protein adhesives -- Enzon
protein C – Baxter Intl., PPL Therapeutics, ZymoGenetics
protein C activator – Gilead Sciences
protein kinase R antags -- NIH
protirelin -- Takeda
protocadherin 2 -- Caprion
Pro-urokinase – Abbott, Bristol-Myers Squibb, Dainippon, Tosoh -- Welfide
P-selectin glycoprotein ligand-1 -- Genetics Institute
pseudomonal infections -- InterMune
Pseudomonas vaccine -- Cytovax
PSGL-Ig -- American Home Products
PSP-94 -- Procyon
PTH 1-34 -- Nobex
Quilimmune-M -- Antigenics
R 744 -- Roche
R 101933
R 125224 -- Sankyo
RA therapy -- Cardion
Rabies vaccine recombinant -- Aventis Pasteur, BioChem Vaccines, Kaketsuken Pharmaceuticals
RadioTheraCIM -- YM BioSciences
Ramot project No. 1315 -- Ramot
Ramot project No. K-734A -- Ramot
Ramot project No. K-734B -- Ramot
Ranibizumab (Anti-VEGF fragment) -- Genentech
RANK -- Immunex
ranpirnase -- Alfacell
ranpirnase-anti-CD22 MAb -- Alfacell
RANTES inhibitor -- Milan
RAPID drug delivery systems -- ARIAD
rasburicase -- Sanofi
rBPI-21, topical -- XOMA
RC 529 -- Corixa
rCFTR -- Genzyme Transgenics
RD 62198
rDnase -- Genentech
RDP-58 -- SangStat
RecepTox-Fce -- Keryx
RecepTox-GnRH – Keryx, MTR Technologies
RecepTox-MBP – Keryx, MTR Technologies
recFSH -- Akzo Nobel, Organon
REGA 3G12
Regavirumab -- Teijin
relaxin -- Connetics Corp
Renal cancer vaccine -- Macropharm
repifermin -- Human Genome Sciences
Respiratory syncytial virus PFP-2 vaccine -- Wyeth-Lederle
Respiratory syncytial virus vaccine – GlaxoSmithKline, Pharmacia, Pierre Fabre
Respiratory syncytial virus vaccine inactivated
Respiratory syncytial virus-parainfluenza virus vaccine -- Aventis Pasteur, Pharmacia
Reteplase -- Boehringer Mannheim, Hoffman La-Roche
Retropep -- Retroscreen
RFB4 (dsFv) PE38
RFI 641 -- American Home Products
RFTS -- UAB Research Foundation
RG 12986 -- Aventis Pasteur
RG 83852 -- Aventis Pasteur
RG-1059 -- RepliGen
rGCR -- NIH
rGLP-1 -- Restoragen
rGRF -- Restoragen
rh Insulin – Eli Lilly
RHAMM targeting peptides -- Cangene
rHb1.1 – Baxter Intl.
rhCC10 -- Claragen
rhCG -- Serono
Rheumatoid arthritis gene therapy

FIG. 28Y

Rheumatoid arthritis vaccine -- Veterans Affairs Medical Center
rhLH -- Serono
Ribozyme gene therapy -- Genset
Rickettsial vaccine recombinant
RIGScan CR -- Neoprobe
RIP-3 -- Rigel
Rituximab -- Genentech
RK-0202 -- RxKinetix
RLT peptide -- Esperion
rM/NEI -- IVAX
rmCRP -- Immtech
RN-1001 -- Renovo
RN-3 -- Renovo
RNAse conjugate -- Immunomedics
RO 631908 -- Roche
Rotavirus vaccine -- Merck
RP 431 -- DuPont Pharmaceuticals
RP-128 -- Resolution
RPE65 gene therapy –
RPR 110173 -- Aventis Pasteur
RPR 115135 -- Aventis Pasteur
RPR 116258A -- Aventis Pasteur
rPSGL-Ig -- American Home Products
r-SPC surfactant -- Byk Gulden
RSV antibody -- Medimmune
Ruplizumab -- Biogen
rV-HER-2/neu -- Therion Biologics
SA 1042 -- Sankyo
sacrosidase – Orphan Medical
Sant 7
Sargramostim -- Immunex
saruplase -- Gruenenthal
Satumomab -- Cytogen
SB 1 -- COR Therapeutics
SB 207448 -- GlaxoSmithKline
SB 208651 -- GlaxoSmithKline
SB 240683 -- GlaxoSmithKline
SB 249415 -- GlaxoSmithKline
SB 249417 -- GlaxoSmithKline
SB 6 -- COR Therapeutics
SB RA 31012 –
SC 56929 -- Pharmacia
SCA binding proteins – Curis, Enzon
scFv(14E1)-ETA Berlex Laboratories, Schering AG
ScFv(FRP5)-ETA –
ScFv6C6-PE40 –
SCH 55700 -- Celltech
Schistosomiasis vaccine -- Glaxo Wellcome/Medeva, Brazil
SCPF -- Advanced Tissue Sciences
scuPA-suPAR complex -- Hadasit
SD-9427 -- Pharmacia
SDF-1 -- Ono
SDZ 215918 -- Novartis
SDZ 280125 -- Novartis
SDZ 89104 -- Novartis
SDZ ABL 364 -- Novartis
SDZ MMA 383 -- Novartis
Secretin – Ferring, Repligen
serine protease inhibs -- Pharis
sermorelin acetate -- Serono
SERP-1 -- Viron
sertenef -- Dainippon
serum albumin, Recombinant human -- Aventis Behring
serum-derived factor -- Hadasit
Sevirumab -- Novartis
SGN 14 -- Seatle Genetics
SGN 15 -- Seatle Genetics
SGN 17/19 -- Seatle Genetics
SGN 30 -- Seatle Genetics
SGN-10 -- Seatle Genetics
SGN-11 -- Seatle Genetics
SH 306 -- DuPont Pharmaceuticals
Shanvac-B -- Shantha
Shigella flexneri vaccine – Avant, Acambis, Novavax
Shigella sonnei vaccine –
sICAM-1 -- Boehringer Ingelheim
Silteplase -- Genzyme

FIG. 28Z

SIV vaccine – Endocon, Institut Pasteur
SK 896 -- Sanwa Kagaku Kenkyusho
SK-827 -- Sanwa Kagaku Kenkyusho
Skeletex -- CellFactors
SKF 106160 -- GlaxoSmithKline
S-nitroso-AR545C –
SNTP -- Active Biotech
somatomedin-1 – GroPep, Mitsubishi-Tokyo, NIH
somatomedin-1 carrier protein -- Insmed
somatostatin -- Ferring
Somatotropin/
Human Growth Hormone -- Bio-Tech. General, Eli Lilly
somatropin -- Bio-Tech. General, Alkermes, ProLease, Aventis Behring, Biovector, Cangene, Dong-A, Eli Lilly, Emisphere, Enact, Genentech, Genzyme Transgenics, Grandis/InfiMed, CSL, InfiMed, MacroMed, Novartis, Novo Nordisk, Pharmacia Serono, TranXenoGen
somatropin derivative -- Schering AG
somatropin, AIR -- Eli Lilly
Somatropin, inhaled -- Eli Lilly/Alkermes
somatropin, Kabi -- Pharmacia
somatropin, Orasome -- Novo Nordisk
Sonermin -- Dainippon Pharmaceutical
SP(V5.2)C -- Supertek
SPf66
sphingomyelinase -- Genzyme
SR 29001 -- Sanofi
SR 41476 -- Sanofi
SR-29001 -- Sanofi
SS1(dsFV)-PE38 -- NeoPharm
ß2 microglobulin -- Avidex
ß2-microglobulin fusion proteins -- NIH
ß-amyloid peptides -- CeNeS
ß-defensin -- Pharis
Staphylococcus aureus infections -- Inhibitex/ZLB
Staphylococcus aureus vaccine conjugate -- Nabi
Staphylococcus therapy -- Tripep
Staphylokinase – Biovation, Prothera, Thrombogenetics
Streptococcal A vaccine -- M6 Pharmaceuticals, North American Vaccine
Streptococcal B vaccine -- Microscience
Streptococcal B vaccine recombinant -- Biochem Vaccines
Streptococcus pyogenes vaccine
STRL-33 -- NIH
Subalin -- SRC VB VECTOR
SUIS -- United Biomedical
SUIS-LHRH -- United Biomedical
SUN-E3001 -- Suntory
super high affinity monoclonal antibodies -- YM BioSciences
Superoxide dismutase – Chiron, Enzon, Ube Industries, Bio-Tech, Yeda
superoxide dismutase-2 -- OXIS
suppressin -- UAB Research Foundation
SY-161-P5 -- ThromboGenics
SY-162 -- ThromboGenics
Systemic lupus erythematosus vaccine -- MedClone/VivoRx
T cell receptor peptides -- Xoma
T cell receptor peptide vaccine
T4N5 liposomes -- AGI Dermatics
TACI, soluble -- ZymoGenetics
targeted apoptosis -- Antisoma
tasonermin -- Boehringer Ingelheim
TASP
TASP-V
Tat peptide analogues -- NIH
TBP I -- Yeda
TBP II
TBV25H -- NIH
Tc 99m ior cea1 -- Center of Molecular Immunology
Tc 99m P 748 -- Diatide

FIG. 28AA

Tc 99m votumumab -- Intracell
Tc-99m rh-Annexin V – Theseus Imaging
teceleukin -- Biogen
tenecteplase -- Genentech
Teriparatide -- Armour Pharmaceuticals, Asahi Kasei, Eli Lilly
terlipressin -- Ferring
testisin -- AMRAD
Tetrafibricin -- Roche
TFPI -- EntreMed
tgD-IL-2 -- Takeda
TGF-Alpha -- ZymoGenetics
TGF-ß -- Kolon
TGF-ß2 -- Insmed
TGF-ß3 -- OSI
Thalassaemia gene therapy -- Crucell
TheraCIM-h-R3 -- Center of Molecular Immunology, YM BioSciences
Theradigm-HBV -- Epimmune
Theradigm-HPV -- Epimmune
Theradigm-malaria -- Epimmune
Theradigm-melanoma -- Epimmune
TheraFab – Antisoma
ThGRF 1-29 -- Theratechnologies
ThGRF 1-44 -- Theratechnologies
Thrombin receptor activating peptide -- Abbott
thrombomodulin – Iowa, Novocastra
Thrombopoietin -- Dragon Pharmaceuticals, Genentech
thrombopoietin, Pliva -- Receptron
Thrombospondin 2 –
thrombostatin -- Thromgen
thymalfasin -- SciClone
thymocartin – Gedeon Richter
thymosin Alpha1 -- NIH
thyroid stimulating hormone -- Genzyme
tICAM-1 -- Bayer
Tick anticoagulant peptide -- Merck
TIF -- Xoma
Tifacogin – Chiron, NIS, Pharmacia
Tissue factor -- Genentech
Tissue factor pathway inhibitor
TJN-135 -- Tsumura
TM 27 -- Avant
TM 29 -- Avant
TMC-151 – Tanabe Seiyaku
TNF tumour necrosis factor -- Asahi Kasei
TNF Alpha -- CytImmune
TNF antibody -- Johnson & Johnson
TNF binding protein -- Amgen
TNF degradation product -- Oncotech
TNF receptor -- Immunex
TNF receptor 1, soluble -- Amgen
TNF Tumour necrosis factor-alpha -- Asahi Kasei, Genetech, Mochida
TNF-Alpha inhibitor -- Tripep
TNFR:Fc gene therapy – Targeted Genetics
TNF-SAM2
ToleriMab -- Innogenetics
Toxoplasma gondii vaccine -- GlaxoSmithKline
TP 9201 -- Telios
TP10 -- Avant
TP20 -- Avant
tPA -- Centocor
trafermin -- Scios
TRAIL/Apo2L -- Immunex
TRAIL-R1 MAb – Cambridge Antibody Technologies
transferrin-binding proteins -- CAMR
Transforming growth factor-beta-1 -- Genentech
transport protein -- Genesis
Trastuzumab -- Genetech
TRH -- Ferring
Triabin -- Schering AG
Triconal
Triflavin
troponin I -- Boston Life Sciences
TRP-2^ -- NIH
trypsin inhibitor -- Mochida

FIG. 28BB

TSP-1 gene therapy –
TT-232
TTS-CD2 -- Active Biotech
Tuberculosis vaccine -- Aventis Pasteur, Genesis
Tumor Targeted Superantigens -- Active Biotech -- Pharmacia
tumour vaccines -- PhotoCure
tumour-activated prodrug antibody conjugates -- Millennium/ImmunoGen
tumstatin -- ILEX
Tuvirumab -- Novartis
TV-4710 – Teva
TWEAK receptor -- Immunex
TXU-PAP
TY-10721 – TOA Eiyo
Type I diabetes vaccine -- Research Corp
Typhoid vaccine CVD 908
U 143677 -- Pharmacia
U 81749 -- Pharmacia
UA 1248 -- Arizona
UGIF -- Sheffield
UIC 2
UK 101
UK-279276 – Corvas Intl.
urodilatin -- Pharis
urofollitrophin -- Serono
Urokinase -- Abbott
uteroferrin-- Pepgen
V 20 -- GLYCODesign
V2 vasopressin receptor gene therapy
vaccines -- Active Biotech
Varicella zoster glycoprotein vaccine -- Research Corporation Technologies
Varicella zoster virus vaccine live -- Cantab Pharmaceuticals
Vascular endothelial growth factor – Genentech, University of California
Vascular endothelial growth factors – R&D Systems
vascular targeting agents -- Peregrine
vasopermeation enhancement agents -- Peregrine
vasostatin -- NIH
VCL -- Bio-Tech. General
VEGF – Genentech, Scios
VEGF inhibitor -- Chugai
VEGF-2 -- Human Genome Sciences
VEGF-Trap -- Regeneron
viscumin, recombinant -- Madaus
Vitaxin
Vitrase -- ISTA Pharmaceuticals
West Nile virus vaccine -- Bavarian Nordic
WP 652
WT1 vaccine -- Corixa
WX-293 -- Wilex BioTech.
WX-360 -- Wilex BioTech.
WX-UK1 -- Wilex BioTech.
XMP-500 -- XOMA
XomaZyme-791 -- XOMA
XTL 001 -- XTL Biopharmaceuticals
XTL 002 -- XTL Biopharmaceuticals
yeast delivery system -- GlobeImmune
Yersinia pestis vaccine
YIGSR-Stealth -- Johnson & Johnson
Yissum Project No. D-0460 -- Yissum
YM 207 -- Yamanouchi
YM 337 -- Protein Design Labs
Yttrium-90 labelled biotin
Yttrium-90-labeled anti-CEA MAb T84.66 –
ZD 0490 – AstraZeneca
ziconotide -- Elan
ZK 157138 -- Berlex Laboratories
Zolimomab aritox
Zorcell -- Immune Response
ZRXL peptides -- Novartis

FIG. 28CC